1 **An Account of the Taxonomy of North American Wolves from Morphological and Genetic**

2 **Analyses**

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47   **Abstract**

48   The available scientific literature was reviewed to assess the taxonomic standing of North

49   American wolves, including subspecies of the gray wolf, *Canis lupus*. The recent scientific

50   proposal that the eastern wolf, *Canis lupus lycaon*, is not a subspecies of gray wolf, but a full

51   species, *Canis lycaon*, is well-supported by both morphological and genetic data and should be

52   accepted. This species' range extends westward to Minnesota, and it hybridizes with gray

53   wolves where the two species are in contact in eastern Canada, the Upper Peninsula of Michigan,

54   Wisconsin, and Minnesota. Genetic data support a close relationship between eastern wolf and

55   red wolf (*Canis rufus*), but do not support the proposal that they are the same species; it is more

56   likely that they evolved independently from different lineages of a common ancestor with

57   coyotes. The Mexican wolf (*Canis lupus baileyi*) is a well-supported subspecies. The available

58   genetic and morphometric data do not provide clear support for the recognition of the Arctic

59   wolf (*Canis lupus arctos*), but the available genetic data are almost entirely limited to one group

60   of genetic markers (microsatellite DNA) and are not definitive on this question. The northern

61   timber wolf (*Canis lupus occidentalis*) and the plains wolf (*Canis lupus nubilus*) are valid

62   subspecies. Their recognition is supported by morphological data and extensive studies of

63   microsatellite DNA variation where both subspecies are in contact in Canada. The wolves of

64   coastal areas in southeastern Alaska and British Columbia should be assigned to *Canis lupus*

65   *nubilus*. There is scientific support for the taxa recognized here, but delineation of exact

66   geographic boundaries presents challenges. Rather than sharp boundaries between taxa,

67   boundaries should generally be thought of as intergrade zones of variable width.

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Introduction

72   The taxonomy of North American members of the genus *Canis* has a complicated and

73   controversial history. This is not surprising in light of their variability in size, proportions, and

74   pelage; large geographic ranges; tendency of various forms to interbreed; and their extirpation

75   over large areas beginning early in the period of colonization by Europeans. Members of North

76   American *Canis*, exclusive of coyotes (*Canis latrans*), are commonly referred to as "wolves."

77   For these North American wolves, 31 published names for subspecies or species are available

78   (Hall and Kelson 1959, Table 1 of this paper). The two most recent comprehensive taxonomic

79   reviews based on morphology both recognize two species, *Canis lupus* (gray wolf) and *Canis*

80   *rufus* (red wolf), but differ in that they recognize as many as 27 (Hall [1981], based primarily on

81   Goldman [1944]) or as few as eight subspecies (Nowak 1995) for the two species collectively.

82   The first of many studies of *Canis* using molecular genetic markers (Wayne and Jenks

83   1991, Lehman et al. 1991) raised new challenges to the general taxonomic scheme (Goldman

84   1944) that had stood for almost 50 years. In particular the possible role of coyotes in the

85   ancestry of both the red wolf and what had been considered gray wolves in the Great Lakes

86   region generated new controversy. Development of even more powerful genetic markers has led

87   to new, highly controversial interpretations, such as the distinctiveness of wolves of the Great

88   Lakes region from gray wolves and the possibility that they are conspecific with red wolves

89   (Wilson et al. 2000), a proposal rejected by others based on genetics (e.g., Koblmüller et al.

90   2009a) and morphometrics (e.g., Nowak 2009). Other controversies include whether the current

91   Great Lakes wolf population is evolutionarily representative of the historical population

92   (Leonard and Wayne 2008), the taxonomic identity of Minnesota wolves (Nowak 2009), the

93   historical northern boundary of the Mexican wolf (*Canis lupus baileyi*) (Leonard et al. 2005),

94   and the taxonomic identity of wolves of Pacific coastal regions (Muñoz-Fuentes et al. 2009).

95   The extreme lack of consensus among researchers on so many important issues related to

96   the taxonomy of North American wolves prompted the present review.

97

98   **Scope and intent**

99   The purpose of this review is to explore the scientific support in the currently available

100   scientific literature for: (1) recognizing any taxonomic subdivisions, including species and

101   subspecies, of North American wolves; (2) recommending at least general geographic boundaries

102   for any recognized taxa, either species or subspecies; and (3) recommending additional research

103   and analysis that would improve the scientific basis for evaluating the taxonomy of wolves.

104   This review provides the authors' views only on whether the validity of each taxon is

105   supported by a preponderance of evidence from the relevant, available scientific literature. It is

106   important to emphasize the following points about the scope of this review:

107   (1) It is an evaluation and synthesis of the available scientific literature. It is not intended

108   to generate and report results of new research.

109   (2) It does not evaluate or make any recommendation on whether any subspecies that is

110   found to be valid should be used as a management unit, as the object of management action, or

111   preferred to an alternative legal classification for protection, such as a distinct vertebrate

112   population segment recognized under the Endangered Species Act (USFWS and NOAA 1995).

113   Suitability of a subspecies as a unit for any of these purposes requires further, separate analysis

114   weighing legal and policy considerations.

115   (3) It is not a review of the conservation status of any of the taxa considered; as such, it

116   does not review threats to or the population status of any entity; and

117   (4) It represents the views of the authors and not necessarily those of the U.S. Fish and

118   Wildlife Service.

119

120   **Species concepts and criteria**

121   There is no single species concept or set of criteria accepted by all taxonomists.

122   Phylogenetic relationships and reproductive relationships represent two major approaches to

123   defining species, but there have been attempts to combine them in identifying species and

124   species limits. Brief descriptions of some general approaches are provided below.

125   (1) The biological species concept (BSC): This concept is based on reproductive

126   relationships among populations. The ability to interbreed and realize gene flow between two

127   populations is the indication that they belong to the same species. The concept is most

128   commonly associated with Mayr (1963, 1970), but has antecedents during the development of

129   evolutionary biology in the 20th century. According to a brief definition by Mayr (1970), a

130   species is a "reproductively isolated aggregate of interbreeding populations." A major difficulty

131   in applying the BSC is encountered when assessing allopatric populations, where reproductive

132   relationships cannot be assessed directly and must be inferred from other information.

133   (2) Phylogenetic species concepts (PSC): Species are identified by their genealogical or

134   phylogenetic relationships and diagnosability. The many variations of these concepts and others

135   are reviewed by Wiley (1981), Avise (2004), and Coyne and Orr (2004).

136   (3) Avise and Ball (1990, Avise 2004) proposed an integration of concepts from the BSC

137   and PSC into "concordance principles." Their approach accepts intrinsic reproductive barriers as

138 basic to species recognition, but incorporates "evidence of concordant phylogenetic partitions at

139 multiple independent genetic attributes."

140   Establishing species limits by assessing reproductive barriers according to the BSC and

141 concordance principles does not require absolute reproductive isolation for recognition of species

142 limits and boundaries. Mayr (1942) provided many examples of inter-specific hybridization,

143 including species of *Canis*, and recognized that there may only be occasional hybrids, or areas

144 where hybridization is common within hybrid zones. He recognized that the stability of some

145 hybrid zones was important in maintaining the overall distinctness of the species involved, and

146 that different habitat preferences are among the mechanisms that can contribute to the stability of

147 hybrid zones. Inter-specific hybridization is now known to be more frequent than understood at

148 the time of the development of the BSC, and it is the reproductive fate of hybrid individuals that

149 is important in determining whether introgression is occurring to the extent that the formerly

150 separate gene pools are merging (Coyne and Orr 2004).

151   (4) The cohesion species concept was proposed by Templeton (1989) to at least partly

152 deal with situations such as those in canids where there is naturally-occurring hybridization

153 among species and reproductive isolation is difficult to evaluate. He provided this definition:

154 "The cohesion species concept is the most inclusive population of individuals having the

155 potential for phenotypic cohesion through intrinsic cohesion mechanisms." Cohesion

156 mechanisms include promoting genetic identity with gene flow and constraints from selective,

157 ecological, developmental, and historical factors.

158   (5) Some recent taxonomists (e.g., Sites and Marshall 2004, de Queiroz 2007) have

159 distinguished between species concepts and the operational criteria for empirically determining

160 species limits, or delimiting species. A "separately evolving metapopulation lineage" has been

161   suggested by de Queiroz (2007) as a feature common to all species concepts, with the criteria

162   from various concepts serving as operational criteria for assessing lineage separation.

163   Operational criteria include fixation of character states, correlated divergence between

164   morphology and genetics or between different genetic marker systems, character divergence,

165   monophyly, diagnosability, ecological divergence, and behavioral differences. Different

166   operational criteria can lead to different conclusions because their necessary properties for

167   species diagnosis develop at different times during the process of lineage divergence and

168   speciation (de Queiroz 2007). Sites and Marshall (2004) and de Queiroz (2007) advocate an

169   eclectic approach that uses the appropriate operational criteria for all available classes of

170   scientific information.

171

172   **Subspecies concepts**

173   There is no scientific consensus on what constitutes a subspecies, and some authorities

174   (e.g., Wilson and Brown 1953, Zink 2003) have questioned the utility of the subspecies level of

175   classification. Following is a description of various subspecies criteria that have been proposed

176   and applied in the taxonomic literature. Because some criteria are more stringent than others, a

177   putative subspecies may meet the criteria and be recognized following one concept, but found to

178   be invalid under a more stringent concept.

179   Nowak (1995, p. 394) discussed the standards he used in revising the subspecies of *Canis*

180   *lupus*: "My investigation largely disregarded such questions [concerning use of very localized

181   characters] and concentrated on general trends in measurable size and proportion that could be

182   evaluated on a continent-wide or worldwide basis. Substantive statistical breaks in such trends,

183   as discussed above, were taken as evidence of taxonomic division."

184   In *The Mammals of North America* (Second Edition), Hall (1981, p. viii) included the

185   following in his "Criteria for Species versus Subspecies." "If crossbreeding occurs in nature at a

186   place or places where the geographic ranges of two kinds of mammals meet, the two kinds are to

187   be treated as subspecies of one species. If no crossbreeding occurs, the two kinds are to be

188   regarded as two distinct, full species."

189   Mayr (1963, glossary) defined subspecies as: "An aggregate of local populations of a

190   species inhabiting a geographic subdivision of the range of the species, and differing

191   taxonomically from other populations of the species." Mayr (1963, page 348) explains

192   "differing taxonomically:" 'Therefore subspecies are to be named only if they differ

193   "taxonomically," that is, by diagnostic morphological characters.' Mayr (1969, p. 190) describes

194   a quantitative method for determining whether populations differ taxonomically: 'A so-called

195   75-percent rule is widely adopted. According to this, a population is recognized as a valid

196   subspecies if 75 percent of the individuals differ from "all" (= 97 percent) of the individuals of a

197   previously recognized subspecies. At the point of intersection between the two curves where this

198   is true, about 90 percent of population A will be different from about 90 percent of the

199   individuals of population B (to supply a symmetrical solution).'

200   Patten and Unitt (2002, p. 27) define subspecies as: "diagnosable clusters of populations

201   of biological species occupying distinct geographic ranges." They do not require that

202   diagnosability be absolute, but advocate 90 percent separation as a more stringent criterion than

203   the 75-percent rule."

204   Avise (2004, p. 362) attempted to incorporate phylogenetic information within a

205   biological species concept in providing the following guidance on recognizing subspecies:

206   'Within such units [=species], "subspecies" warranting formal recognition could then be

207   conceptualized as groups of actually or potentially interbreeding populations (normally mostly

208   allopatric) that are genealogically highly distinctive from, but reproductively compatible with,

209   other such groups. Importantly, the empirical evidence for genealogical distinction must come,

210   in principle, from concordant genetic partitions across multiple, independent, genetically based

211   molecular (or phenotypic; Wilson and Brown 1953) traits.'

212   The most stringent criterion that has been proposed for subspecies recognition is

213   reciprocal monophyly (Zink 2004). Application of a monophyly criterion requires that all

214   individuals in a taxon be genealogically closer to one another than to any individual in another

215   taxon. A number of objections to monophyly as a subspecies criterion have been raised, perhaps

216   foremost is that in phylogenetic classifications it is a species-level criterion and inappropriate for

217   application below the species level (Goldstein et al. 2000, Patten and Unitt 2002). Its application

218   using genetic data is limited to genetic sequences that do not recombine, such as mitochondrial

219   DNA, and therefore usually depends on one type of marker rather than multiple markers that can

220   be tested for concordance, as in the Avise (2004) criterion. In addition, there are many instances

221   of related species that have achieved reproductive isolation but are not reciprocally monophyletic

222   (Avise 2004); it takes many generations (on the order of four to seven times the effective

223   population size) after putative taxa are separated for reciprocal monophyly to be achieved

224   (Hudson and Coyne 2002).

225   A common feature of all of the above definitions is that they recognize that subspecies

226   are groups of populations, and most recognize that subspecies can be variable and overlap in

227   distinguishing characters to some degree.

228

229   **Limitations of the available data**

230   Several factors concerning the available scientific information bearing on wolf

231   relationships complicate the assessment of taxonomic relationships.

232   Wolves have been extirpated over large portions of North America, particularly most of

233   the conterminous United States (Figure 1), so there are large gaps in geographic coverage,

234   particularly for genetic data. Recent studies (discussed in later sections of this review) of DNA

235   markers from museum specimens have attempted to address these gaps, but as yet they represent

236   relatively few individuals.

237   For evaluating continent-wide patterns of variation and their potential taxonomic

238   implications, it would be ideal to have comprehensive sampling across the landscape. This

239   would allow for more rigorous testing and formulation of evolutionary scenarios, and for

240   application of increasingly sophisticated methods of landscape genetics. Regrettably, sampling

241   of wolf populations is far from even over North America. Sampling patterns can influence the

242   interpretation of the genetic structure of populations and lead to erroneous conclusions (Schwartz

243   and McKelvey 2009). Sampling may be relatively intensive in areas that still have large wolf

244   populations, such as Alaska and northern Canada, but information on other areas may be limited

245   to a few, widely spaced individuals. Some published studies (e.g., Koblmüller et al. 2009a)

246   report results from large data sets, but without sufficiently explicit geographic information to

247   permit the reader to evaluate genetic population structure and interactions among populations.

248   Comparable sets of data are not available for many areas of taxonomic interest. For

249   example, some areas may have detailed data on microsatellite DNA variation, but lack

250   information about lineage markers (mitochondrial DNA and Y-chromosome variation).

251   Very few of the reviewed studies were designed to address taxonomic questions. Studies

252   designed for other purposes may not be informative on specific taxonomic issues and the

253   evaluation of putative taxa.

254   The nature of the available data do not permit the application of many of the subspecies

255   criteria reviewed above. For example, the "75-percent rule" is for individual character analysis,

256   but most available analyses of morphological data for wolves use multivariate statistics that

257   summarize variation in many characters. Furthermore, the available data on a particular

258   taxonomic question comprise a variety of very different types of information that must be

259   integrated. The approach to subspecies of Avise (2004), described above, is the most applicable

260   to the disparate data sets available on wolves.

261   For decades the subspecies classification of gray wolves, *Canis lupus*, was the 24

262   subspecies recognized by Hall and Kelson (1959) and Hall (1981), which was largely based on

263   the work of Goldman (1937, 1944). There is very little information for some of these named

264   subspecies, especially from genetic studies. Nowak (1995) has reduced the number of

265   recognized subspecies to five; the subspecies and their geographic ranges recognized by Nowak

266   (1995, 2002) are presented in Figure 2 of this paper.

267

268   **Approach taken in this review**

269   *Species limits*. Certain attributes of North American *Canis* present special challenges in

270   evaluating species-level taxa, including the ability of different forms to hybridize under certain

271   conditions. There is also a temporal dimension to wolf relationships; some lineages that

272   maintained their distinctiveness from one another are now in secondary contact where earlier

273   ecological and other factors that had formerly inhibited hybridization have been altered. On the

274   other hand, there is detailed genetic information from some areas, and information on variation

275   in ecology and behavior is available for explaining historic and modern relationships of different

276   populations.

277   This review generally follows concordance principles in recognizing species (Avise and

278   Ball 1990, Avise 2004). This is an eclectic approach that seeks to identify species as separate

279   lineages supported by concordant data from various classes of genetic markers, morphometric

280   analysis, behavior, and ecology. This approach is appropriate for North American *Canis* because

281   populations of the putative species are or have been in contact with one another and there is

282   considerable genetic information bearing on reproductive relationships. At the same time, there

283   are extensive data from genetic lineage markers (mitochondrial DNA and Y-chromosome

284   haplotypes) that provide phylogenetic information that needs to be considered to understand the

285   evolutionary history, ancestral condition, and taxonomic relationships of North American wolf

286   populations. Lineage markers are essential for inferring possible pre-contact population

287   differences that became complicated by contact and admixture between formerly separate

288   populations or lineages (Cathey et al. 1998, Hannote et al. 2000, Feng et al. 2000, Pidancier et al.

289   2006).

290   As discussed later in this review, North American *Canis* comprise two major lineages or

291   clades: one including most gray wolves, and the other includes eastern wolf, red wolf, and

292   coyote. The species-level taxonomic implications of these two clades is first evaluated. With

293   respect to *Canis lupus*, the most controversial question about its species limits raised subsequent

294   to Goldman's (1937, 1944) consolidation of various North American species names (discussed in

295   the later section on taxonomic background) under *C. lupus* is the proposal that the eastern wolf,

296   *Canis lupus lycaon* is a separate species and outside the species limits of *C. lupus*. Reproductive

297   relationships of populations representing the eastern wolf and other *C. lupus,* in the narrow

298   sense, can be assessed because there has been interbreeding and admixture, and data are

299   available from genetic markers suitable for evaluating the extent of admixture and alternative

300   interpretations of the origin of coyote clade markers within the eastern wolf. Whether the red

301   wolf is within the species limits of *C. lupus* is less controversial.

302   Because there has been at least historical contact and informative genetic data are

303   available, a similar approach can be taken in evaluating species limits within the coyote clade,

304   with one exception. Extirpation, limited geographic sources for genetic data, and uncertainties

305   about historical distributions do not permit a meaningful assessment of reproductive

306   relationships between eastern wolf and red wolf. For determining species-level relationships

307   between these two putative taxa, they must essentially be treated as allopatric populations, and

308   operational criteria other than reproductive relationships must be applied. Some data are

309   available for assessing non-reproductive criteria such as correlated divergence between

310   morphology and genetics, different genetic marker systems, character divergence, cohesion,

311   monophyly, and diagnosability.

312   As discussed previously under species concepts, evaluation of reproductive relationships

313   following concordance principles and the biological species concept does not require absolute

314   reproductive isolation for recognition of species-level differences. If absolute isolation were

315   required, all North American *Canis*, wolves and coyotes, would be considered one species, or a

316   "ring species" (Mayr 1942), because all component taxa are linked by evidence of interbreeding,

317   although the incidence of such effective introgression can range from ancient and very rare in

318   some cases to modern and ongoing in others. Requirement of absolute reproductive isolation as

319   a standard for species delimitation would mean that coyotes and wolves in eastern North

320 America are the same species, despite marked differences in morphology, ecology, behavior, and

321 genetic composition. Such a single-species classification would obscure evolutionarily

322 important diversity. Partially because of the power of new systems of molecular genetic

323 markers, incomplete reproductive isolation between recognized species is now known to be

324 common, especially in certain groups (Grant and Grant 1992, Schwenk et al. 2008), and

325 examples include familiar species such as some species pairs of Darwin's finches (Grant and

326 Grant 2006), mallards (*Anas platyrhynchos*) and American black duck (*Anas rubripes*) (Mank et

327 al. 2004), and Canadian lynx (*Lynx canadensis*) and bobcat (*Lynx rufus*) (Schwartz et al. 2004).

328 The approach of this review following concordance principles will allow full consideration of the

329 available information in evaluating the relationships among populations, the extent of

330 interbreeding, and the likely consequences for recognizing species limits.

331

332 *Subspecies of gray wolf.* The initial approach of this analysis was to compare the distributions of

333 the five subspecies of gray wolf (*Canis lupus*) identified in the most recent taxonomic revision of

334 that species (Nowak 1995) with available scientific information on inter-populational differences

335 and relationships, primarily from studies of genetic variation. The scientific support for the

336 validity of these taxa is evaluated by summarizing the results of each scientific study with a

337 bearing on the taxonomic standing of a particular subspecies and relationships to other

338 subspecies. A subspecies is found to be supported when the geographic distribution of specific

339 genetic markers coincides with its general distribution based on morphological analyses. The

340 distribution of mitochondrial DNA haplotypes associated with different clades, and presumed

341 Old World sources, is of particular interest. This approach most closely resembles the

342 subspecies definition and criteria of Avise (2004) in that concordance between morphometric

343   and genetic data is taken as evidence for the validity of a subspecies. This approach should not

344   be interpreted as *a priori* acceptance of Nowak's (1995) subspecies classification. This is a

345   taxonomic evaluation, and it is reasonable to consider the most recently revised taxonomy, which

346   is that of Nowak (1995). Based on additional information, primarily genetic data, this review

347   comes to conclusions that differ from Nowak (1995) on some taxonomic interpretations, and

348   subspecies that Nowak (1995) reduced to synonymy are considered when additional data suggest

349   that they deserve further evaluation.

350

351   **Biology of the Species**

352   This section first provides summaries of the taxonomic history of *Canis* and some aspects

353   of ecology and behavior that have been identified as important in explaining population

354   structure. The major part of this section comprises summaries of individual studies of

355   morphology and genetics that are relevant to the evaluation of the taxonomy of North American

356   wolves.

357

358   **Taxonomic background on wolf species and subspecies**

359   *History and overview of the genus Canis.* This brief summary of the global history of *Canis* is

360   based primarily on the reviews by Nowak (1979) and Kurtén and Anderson (1980).

361   The genus *Canis* originated in North America by the middle Pliocene. Members of the

362   genus probably began colonizing the Old World soon (in geological time) thereafter, where their

363   descendents include the modern species *Canis adustus* (side-striped jackal; range: Africa), *Canis*

364   *aureus* (golden jackal; Eurasia and North Africa), *Canis mesomelas* (black-backed jackal;

365   Africa), and *Canis simensis* (Ethiopian wolf; Ethiopia) (Wilson and Reeder 2005).

366   Diverse lineages and species of *Canis*, including coyote, evolved in North America

367   during the Pliocene and Pleistocene. Members of one of these North American lineages entered

368   Eurasia in the early Pleistocene and eventually evolved into *Canis lupus*. Gray wolf later entered

369   North America, where its fossils first appear in middle Pleistocene deposits. More than one

370   invasion of North America by Eurasian *C. lupus* has been suggested based on morphological

371   data and biogeographical reconstruction (Nowak 1983, 1995). This has been confirmed by

372   genetic data that support at least three separate invasions from different Eurasian lineages to

373   explain the patterns of genetic variation observed in modern *C. lupus* of North America (Vilà et

374   al. 1999). An additional gray wolf lineage known only from Pleistocene individuals preserved in

375   permafrost in Alaska became extinct without leaving modern descendents (Leonard et al. 2007).

376   Gray wolf is the only species of *Canis* with a range that includes portions of both Eurasia

377   and North America. Gray wolves had very large historic distributions in both areas: throughout

378   all of Eurasia except southeast Asia, and in North America from the Arctic to Mexico. Based on

379   morphometric analyses, Nowak (1995) recognized five subspecies of *C. lupus* in North America.

380   Of the 12 subspecies previously recognized in Eurasia, three were considered synonyms of other

381   subspecies, and there was insufficient material to statistically evaluate four others. None of the

382   modern recognized subspecies occurs or occurred in both Eurasia and North America.

383

384   *Canis in North America.* The first published name of a taxon of *Canis* from North America is

385   *Canis lycaon*, which was published in 1775 based on the earlier description and illustration of an

386   individual that was thought to have been captured near Quebec (Goldman 1937). The next North

387   American taxon names were published when Say (1823) named and described *Canis nubilus*

388   based on wolves he observed in eastern Nebraska.   The coyote (*Canis latrans*) was also

389   described by Say (1823) from the same Nebraska locality at the same time, and his observations

390   appear to be the first that clearly distinguish between wolves and coyotes. These and the other

391   28 available scientific names subsequently described from North American wolf taxa are listed

392   in chronological order in Table 1. Wolf taxa were originally described as either subspecies

393   (sometimes indicated as a trinomial "variety") or species through 1912. Thereafter, all new taxa

394   were described as subspecies. Most available wolf names were subspecies described in the

395   1930s and 1940s.

396   Earlier names were published as individual descriptions in various publications,

397   including reports of exploratory expeditions. The first attempts to compile consolidated

398   treatments of North American wolf taxa were the incomplete reviews by Miller (1912) and

399   Pocock (1935). Goldman (1937, 1944) provided the first truly comprehensive treatment of

400   North American wolf taxa, but did not include coyote. Goldman's classification addressed

401   uncertainties in the nomenclatural history of the taxa, and included many subspecies, many of

402   which he himself described. A particularly notable feature of Goldman's classification was

403   recognition of two species of wolves in North America: red wolf (as *Canis niger*, now known as

404   *Canis rufus*) occupying parts of the southeastern United States, and gray wolf occupying the

405   remaining range of wolves in North America. .

406   For decades the subspecies classification of gray wolves was the 24 subspecies

407   recognized by Hall and Kelson (1959) and Hall (1981), which was based primarily on

408   Goldman's (1944) classification. The range map of subspecies from Goldman (1944, Figure 14)

409   is reproduced here as Figure 3. Nowak's morphometric studies led him to propose the reduction

410   in number of the North American subspecies of gray wolf from the 24 previously recognized to

411   five (Table 2, Figure 2). Brewster and Fritts (1995) summarized controversies concerning North

412 American wolves, with a concentration on western North America, based on the genetic and

413 morphometric information available at that time.

414   The following sections provide more detailed taxonomic background on individual North

415 American wolf taxa.

416

417 *Red wolf.* *Canis rufus* has usually been recognized as a species separate from gray wolf

418 (Goldman 1937, 1944; Nowak 1979; Hall 1981; Baker et al. 2003), but is sometimes considered

419 a subspecies of gray wolf (Lawrence and Bossert 1967, Wilson and Reeder 2005). Nowak

420 (1979, p. 85) has noted that the name *Canis niger* (Bartram, 1791), which was used by Goldman

421 (1944) and some other authors for this species, was determined by the International Commission

422 on Zoological Nomenclature to be unavailable for nomenclatural purposes.

423   The three subspecies of red wolf recognized by Goldman (1937, 1944) and Hall (1981)

424 are listed in Table 3 along with their general historical ranges. The red wolf survives only in

425 captive breeding facilities and reintroduced populations in North Carolina (Phillips et al. 2003).

426 All surviving individuals are descendants of red wolves captured within the historic range of the

427 subspecies *C. r. rufus*, so that nearly all genetic data on *C. rufus* are derived from individuals

428 attributable to that subspecies. Because all living red wolves are derived from this single

429 subspecies, the subspecies classification will not be treated in this review.

430

431 *Gray wolf subspecies.* The more expansive subspecies classification of Goldman (1944) Hall

432 and Kelson (1959), and Hall (1981) as well as the simplified classification of Nowak (1995) are

433 presented in Table 2. The recognized names in this table will be used in the following discussion

434 of their taxonomic treatment.

435   The taxonomic status of the eastern wolf is controversial. It has been considered a full

436   species, *Canis lycaon* (Wilson et al. 2000, Baker et al. 2003); a subspecies of gray wolf, *Canis*

437   *lupus lycaon* (Goldman 1937, 1944; Nowak 1995, 2002, 2003); the result of coyote introgression

438   into gray wolf (Lehman et al 1991); the same species as the red wolf (Wilson et al. 2000); or a

439   result of hybridization between red wolf and gray wolf (Nowak 2002, 2003, 2009).

440   Goldman (1937, 1944) considered the eastern wolf to be a subspecies, *C. l. lycaon*, found

441   from southern Quebec and Ontario to Minnesota. He also described *Canis lupus labradorius*

442   from northern Quebec, stating that it was similar to eastern wolf, but larger. Goldman (1944)

443   mapped a geographic range for eastern wolf that extended from northeast Florida to eastern

444   Minnesota and States to the east, and Ontario and southern Quebec in Canada (Nowak [2002]

445   now places the Florida location at "vicinity of Miami"). He recognized the following three

446   neighboring subspecies:

447   *Canis lupus nubilus* (plains wolf): bordering eastern wolf on the west from southern

448   Illinois to Manitoba. Goldman (1944, p. 444) notes, however, "[s]pecimens from eastern

449   Minnesota and Michigan seem more properly referable to *lycaon*, but relationship to *nubilus* is

450   shown in somewhat intermediate character."

451   *Canis lupus hudsonicus* (Hudson Bay wolf): bordering eastern wolf range west of

452   Hudson Bay in northern Manitoba.

453   *Canis lupus labradorius* (Labrador wolf): bordering eastern wolf range in northern

454   Quebec.

455   The general ranges of these three subspecies were followed by Hall and Kelson (1959) and Hall

456   (1981).

457   Relevant to the boundary of eastern wolf, Standfield (1970) observed that a larger type of

458   wolf occurred in boreal areas north of Lake Superior, and a smaller wolf occurred in deciduous

459   forests to the east and southeast. Mech and Frenzel (1971) suggested that some wolves in

460   northeastern Minnesota were *C. l. nubilus* based on color forms of black and white that Goldman

461   (1944) had reported as common for *C. l. nubilus* but not for eastern wolf.

462   Corresponding to varying views on its taxonomic standing, the geographic range of

463   eastern wolf has not been consistently defined: The very broad ranges in eastern North America

464   recognized by Goldman (1944) and Hall (1981) were reduced by Nowak (1995) to a much

465   smaller area centered on southern Ontario and Quebec.

466   Wilson et al. (2000) proposed that eastern wolf be restored to full species status based on

467   its genetic distinctness from gray wolf. They also proposed that it is the same species as red

468   wolf, and that this combined taxon be recognized under the earlier published name, *Canis*

469   *lycaon.*

470   The Mexican wolf was described by Nelson and Goldman (1929) as *Canis nubilus*

471   *baileyi*, with a type locality identified in Chihuahua, Mexico. Its distribution was described as:

472   "Southern and western Arizona, southern New Mexico, and the Sierra Madre and adjoining

473   tableland of Mexico as far south, at least, as southern Durango." The specimens examined

474   included a wolf from Kendrick Peak on the Coconino Plateau in north-central Arizona and

475   several individuals from the Sacramento Mountains, New Mexico.

476   Goldman (1937) re-classified Mexican wolf as a subspecies of the species *Canis lupus*, so

477   that its name became *Canis lupus baileyi*. He also included the Kendrick Peak, Arizona,

478   specimen with his newly described *Canis lupus mogollonensis* (Goldman 1937, 1944), which

479   shifted the northern limits of Mexican wolf further south in Arizona. Goldman (1933) mapped

480   the eastern boundary of Mexican wolf as contiguous with the western boundary of *C. l.*

481   *monstrabilis* in southeastern New Mexico, far western Texas, and eastern Mexico. This view of

482   the boundary of Mexican wolf in Arizona was followed by Hall and Kelson (1959), Nowak

483   (1979), and Hall (1981).

484   Based on their morphometric analysis of wolves of the southwestern United States and

485   adjacent Mexico, Bogan and Mehlhop (1983) referred wolves formerly assigned to *C. l.*

486   *mogollonensis* and *C. l. monstrabilis* to Mexican wolf.

487   Nowak (1995) included Mexican wolf as one of five North American subspecies that he

488   recognized in his revision of gray wolf subspecies, but contrary to Bogan and Mehlhop (1983),

489   referred *C. l. mogollonensis* and *C. l. monstrabilis* to *C. l. nubilus*.

490   Arctic wolf, *Canis lupus arctos*, was described from skulls from Melville Island and

491   Ellesmere Island in the Canadian Arctic (Pocock 1935). *Canis lupus arctos* was subsequently

492   recognized by Goldman (1944), Hall and Kelson (1959), and Hall (1981).

493   Based on morphometric analysis, Nowak (1995) placed *Canis lupus orion* and *Canis*

494   *lupus bernardi* as synonyms of *C. l. arctos*. Both were recognized as separate subspecies by

495   Goldman (1944), Hall and Kelson (1959), and Hall (1981). The range of Nowak's expanded *C.*

496   *l. arctos* generally includes Greenland and all the Canadian Arctic Islands, except Baffin Island,

497   which was included within the range of *Canis lupus nubilus*.

498   *Canis lupus nubilus* and *Canis lupus occidentalis* are the most geographically widespread

499   of the five subspecies of gray wolf recognized by Nowak (1995), and share long and complex

500   borders. They also have the largest synonymies of the five species, with 11 synonyms recognized

501   for *C. l. nubilus* and six for *C. l. occidentalis* (Table 3).

502

503 **Summary of relevant literature**

504 *Ecology, behavior, prey and habitat.* Based on its extraordinarily large historical range, gray

505 wolf has been one of the most successful large, terrestrial vertebrate species to occupy the earth.

506 In North America, it occurred in nearly all natural types of habitats from the Arctic to tropical

507 Mexico. This section summarizes aspects of habitat, prey, and behavior that have been invoked

508 to explain certain patterns of variation in wolves.

509 In North America, wolves can be successful in all natural habitat types (Carroll 2003,

510 Carroll et al. 2003, 2006, Oakleaf et al. 2006), except the most extreme deserts. Differences in

511 habitat have been correlated with variations in behavior, including migration and prey selection.

512 For example, Kolenosky and Stanfield (1975) have described variation in Ontario wolves, where

513 larger wolves of boreal forests specialize on moose (*Alces americanus*) and caribou (*Rangifer*

514 *tarandus*) as prey, while smaller wolves in deciduous forest habitats specialize on white-tailed

515 deer (*Odicoileus virginiana*). Carmichael et al. (2001) and Musiani et al. (2007) have proposed

516 that differences in migratory behavior and prey have influenced genetic differences between

517 wolves that follow migratory caribou on the tundra and wolves that prey on more sedentary

518 caribou in forested areas. These studies are further discussed in the following sections on

519 morphology and genetics. North American wolves specialize on large mammals as prey. In

520 addition to caribou, moose, and deer, they feed on muskox (*Ovibos moschatus*), American bison

521 (*Bison bison*), elk (*Cervus elaphus*), mountain sheep (*Ovis* sp.), and mountain goat (*Oreamnos*

522 *americanus*). They also consume domestic ungulates: cattle, sheep, and goats. Mech (1974)

523 indicated that American beaver (*Castor canadensis*) are the smallest prey to be consistently

524 reported for wolves. Lagomorphs and smaller rodents are consumed opportunistically.

525   Pack structure, thought to be key to the ability of gray wolves to catch and kill large

526   ungulate prey, has been alternatively explained by kin selection and benefits of sharing prey with

527   offspring. Wolves are cursorial animals capable of traveling long distances (e. g., Mech 1987,

528   Musiani et al. 2007). Wolves can range from one habitat type to another, and are capable

529   swimmers (Mech 1974). Even where rivers are too wide for regular crossing, wolves can cross

530   when sufficient ice forms (Carmichael et al. 2001). Mountains are generally not a barrier to wolf

531   movement, and in some portions of their range, mountains are where wolves are most common.

532   Particularly steep and high ranges have, however, been invoked to explain the partial isolation

533   and genetic divergence of coastal wolves in southeastern Alaska and British Columbia from

534   inland populations (Weckworth et al. 2005, 2010).

535   The factors briefly discussed above have been invoked as ad hoc explanations to explain

536   certain patterns of morphological or genetic variation in wolves. Wolves are large, vagile

537   animals that have few natural limitations in areas that they can colonize. There do not appear to

538   be any general rules predicting where wolves will be found or where geographic variation can be

539   expected. There can, however, be combinations of behavior, prey, and habitat that can

540   contribute to the partial isolation of populations, and foster interpopulational differences.

541   Instances will be identified in the following sections summarizing studies on morphology and

542   genetics.

543

544   *Summaries of relevant studies.* Studies of the morphology and genetics of North American

545   *Canis* are summarized in the following sections, which are organized into categories by the type

546   of data or genetic marker used. Each discussion of a category of information is followed by a

547   brief summary of areas of agreement or disagreement among the studies. The species and

548   subspecies names used in these summaries are those used by the authors of these papers; use of

549   these names in these sections should not be interpreted as our acceptance of any author's

550   nomenclature. Similarly, the conclusions stated in the summaries are those of the author(s) of

551   each paper being summarized. Our analysis and conclusions are presented in the Analysis and

552   Discussion section later in this paper.

553

554   *Morphology.* Nearly all recent studies of morphological variation among taxa of North

555   American *Canis* employed the multivariate statistical methods of principal components analysis,

556   discriminant function analysis, or both. Discriminant function analysis requires that groups be

557   known or distinguished a priori by other data and is most useful for evaluating the affinity of

558   unknown individuals (e. g., Maldonado et al. 2004), and for identifying characters most useful

559   for distinguishing among groups (James and McCulloch 1990). Its use in determining inter-

560   group differences has been criticized because the a priori identification of groups is sometimes

561   based on the same data that are used to generate the distance measures, which introduces

562   circularity into the analysis, and differences that can readily discriminate between groups may be

563   relatively small and of little biological significance (James and McCulloch 1990, Lance et al.

564   2000). For measuring inter-group differences for taxonomic analysis there are more objective

565   methods, such as principal components analysis; an example is discussed later in the analysis of

566   the standing of the Mexican wolf, *Canis lupus baileyi*. In the following summaries of statistical

567   studies of morphology, the a priori groups subjected to discriminant function analysis are

568   identified.

569   Jolicoeur (1959) carried out an analysis of the distribution of coat color and bivariate and

570   multivariate discriminant function analyses of skull features of 499 *C. lupus* from western

571   Canada. Samples were grouped for the discriminant function analyses by regions within

572   Canadian provinces. He found general patterns of the incidence of pale pelage increasing

573   towards the Arctic, and skulls trending from shorter and broader in the northeast to longer and

574   narrower to the southeast portion of the study area. Although the study was not framed in a

575   taxonomic context, he suggested that "far too many subspecific designations are now in use,"

576   referring to the classification of Goldman (1944). A notable result was that the samples from

577   Vancouver Island were more like individuals from further north than to wolves on the

578   neighboring mainland of British Columbia (Jolicoeur 1959, p. 297).

579   In a study of North American canids using discriminant function analysis, Lawrence and

580   Bossert (1967) included a comparison of groups classified as "*Canis lupus*," *Canis rufus* (under

581   the name *Canis niger*), and *Canis lupus lycaon*. The *C. lupus* sample was found to be

582   intermediate between *C. l. lycaon* (from Algonquin Provincial Park, Ontario) and *C. rufus*. The

583   validity of this result is difficult to evaluate because the geographic source of the *C. lupus* sample

584   of 20 wolves was not indicated and "large individuals were avoided," thus biasing the sample.

585   Another factor limiting comparison to subsequent morphometric studies was the determination

586   of character values relative to the length of the skull rather than actual measurements. This

587   removed size as a character, and size is generally considered an important character in evaluating

588   variation among wolves in North America (Kolenosky and Stanfield 1975, Nowak 1979,

589   Schmitz and Kolenosky 1985).

590   Kolenosky and Standfield (1975) studied skulls of two types of wolves from within the

591   Ontario range of *C. l. lycaon* (as broadly defined by Goldman [1944]) using discriminant

592   function analysis and provided comparisons of whole body mass (n = 594) and coat color (n =

593   1404). The samples included 105 "boreal-type" (from areas of boreal forests) wolves and 122

594   "Algonquin-type" (from deciduous forest regions). Over 75% of boreal-type skulls could be

595   distinguished from those of the Algonquin, or eastern wolf, type. Boreal males averaged 34.5 kg

596   compared to 27.5 kg for Algonquin-type males. Coat colors of Algonquin-type individuals were

597   nearly all gray-faun, while boreal-type were mostly gray-faun, but many individuals were black

598   or cream. They suggested that the size and color of boreal-type individuals were more like *C. l.*

599   *nubilus*, and that the two Ontario forms may not be interbreeding. They associated these types of

600   wolves with different ungulate prey species, with the larger boreal-type wolves preying on

601   moose and caribou, and the Algonquin-type wolves on white-tailed deer.

602   Skeel and Carbyn (1977) performed principal components and discriminant function

603   analyses on 311 wolf skulls from widely-spaced localities in central and northern North America,

604   including several Canadian national parks. Samples were grouped by subspecies or by park for

605   discriminant function analysis. For addressing the question of the relationship of *C. l. lycaon* to

606   other *C. lupus*, the relevant samples were from the southwestern corner of Ontario (referred to *C.*

607   *l. lycaon*), historical *C. l. nubilus* (primarily from the north-central United States), *C. l.*

608   *hudsonicus* from southeast Northwest Territories, and *C. l. griseoalbus* from Manitoba and

609   Saskatchewan. The last two subspecies were later treated as synonyms of *C. l. nubilus* and *C. l.*

610   *occidentalis*, respectively, in Nowak's (1995) taxonomic revision, which is discussed later.

611   Strong geographic patterns were not obvious, except that *C. l. lycaon* is generally more similar to

612   *C. l. nubilus* and its synonyms than to samples from further to the northwest, which would

613   probably be attributable to *C. l. occidentalis* in Nowak's (1995) revised classification. Skeel and

614   Carbyn's (1977) general conclusion was that there is large overlap in characters among

615   individuals, but that wolves in "boreal-subalpine forest regions" are larger.

616   In comparing *C. l. occidentalis*, primarily from Canadian national parks, to other

617   subspecies of *C. lupus*, three-dimensional principal components plots for males and females

618   (Skeel and Carbyn 1977, Figure 2 and Figure 3, respectively) showed a clear separation of *C. l.*

619   *occidentalis* (codes W, R. J, and P in the figures) from a grouping that included *C. l. nubilus*, *C.*

620   *l. hudsonicus, and C. l. irremotus* (the latter two are synonyms of *C. l. nubilus* in Nowak's

621   [1995] classification) samples on the first principal component, which can be attributed to the

622   larger size of *C. l. occidentalis* (Skeel and Carbyn's Figure 2 is reproduced here as Figure 4).

623   Discriminant function plots (Skeel and Carbyn 1977, Figure 4 and Figure 5) showed polygons

624   for *C. l. occidentalis* and *C. l. hudsonicus* overlapping minimally, and more substantially

625   between *C. l. occidentalis* and both *C. l. nubilus* and *C. l. irremotus*. Further analysis with

626   clustering (Skeel and Carbyn 1977, Figure 6) showed discontinuity between *C. l. occidentalis*

627   and the other subspecies, which grouped closer together. A multi-dimensional scaling analysis

628   (Skeel and Carbyn 1977, Figure 7) also separated *C. l. occidentalis* and *C. l. nubilus* samples,

629   with the exception that Wood Buffalo National Park *C. l. occidentalis* were closest to *C. l.*

630   *hudsonicus* from Northwest Territories on Hudson Bay; these are the two northern-most areas

631   included in the study.

632   Nowak (1979) reviewed the taxonomic history and carried out discriminant function

633   analyses of North American *Canis*. Groups for the initial analysis were gray wolf, red wolf,

634   coyote, and domestic dog. Various samples representing specific populations, time periods when

635   specimens were collected, or extinct species of *Canis* were then plotted and compared to the

636   positions of the samples from the initial analysis. Of particular relevance to the present review

637   was his treatment of the relationships and taxonomic standing of *C. rufus* and *C. l. lycaon*.

638   Nowak (1979, p. 87) found little statistical overlap between early (before extensive

639   introgression by coyotes) *C. rufus* (n = 74 males, 55 females) and western *C. lupus* (n = 233

640   males, 146 females) skulls, although a few specimens were difficult to assign. He noted (p. 29)

641   that *C. rufus* resembled *C. l. lycaon* more than it did any other subspecies of *C. lupus*.

642   Nowak (1979, Figure 7) found substantial, but not complete statistical overlap between

643   skulls of *C. l. lycaon* and other *Canis lupus* from western North America as *Canis lupus lycaon*

644   individuals were generally smaller. Nor were the boreal type (Ontario type of Standfield [1970])

645   wolves of Michigan, Minnesota, Wisconsin, and western Ontario and the deciduous type

646   (Algonquin type of Standfield [1970]) of southeastern Ontario and southern Quebec sharply

647   delineated. Some characters in wolves from the western range of *C. l. lycaon* were found to be

648   intermediate between the eastern *C. l. lycaon* and *C. l. nubilus* from the Great Plains (Nowak

649   1979, p. 20), thus lending some support to Mech and Frenzel's (1971) suggestion that some

650   eastern Minnesota wolves were *C. l. nubilus*. Nowak's (1979, p. 21) general conclusion was that

651   individuals that he referred to *C. l. lycaon* were no more distinctive than other subspecies of *C.*

652   *lupus*.

653   Nowak (1983, Figure 6) performed a preliminary bivariate analysis of skulls of various

654   subspecies of North American *C. lupus*, generally following Goldman's (1944) classification.

655   The two studied characters generally reflect the length and width of skulls. This analysis

656   indicated a cline in the two characters, with size increasing from south to north in central North

657   America, but with a break or discontinuity at approximately the U.S.-Canada border in central

658   North America that divided southern and northern *C. lupus*.

659   Based on these data, he proposed new geographic groupings of North American wolves.

660   In this scheme (Nowak 1983, Figure 7b), wolves from Minnesota, Wisconsin, the Upper

661 Peninsula of Michigan, and southwestern Ontario were grouped with southern wolves of the U.S.

662 Great Plains to the west, rather than with *C. l. lycaon* as in Goldman (1944). The Hudson Bay

663 sample (*C. l. hudsonicus*) was also grouped with the southern wolves. The sample of *C. l.*

664 *columbianus* of far western Canada was intermediate but closer to the northern group.

665   Skulls from the Canadian Arctic Islands (subspecies *Canis lupus arctos* and *C. l.*

666 *bernardi*) were outliers to this general north-south trend in overall size and were distinguished by

667 having skulls that were wide relative to their length. Nowak (1983, Figure 7a, b) suggested that

668 at the maximum extent of Pleistocene glaciations, the ancestors of *Canis lupus arctos* were

669 isolated north of the ice sheet in a refugium in northern Greenland, and then spread westward to

670 the Arctic Islands following withdrawal of the glaciers. He also speculated that *C. l. bernardi*

671 and *C. l. orion*, an Arctic subspecies from Greenland, may have declined and their former ranges

672 occupied by *C. l. arctos.*

673   Based on this information and historical factors, Nowak (1983, Figure 7a, b) suggested a

674 "hypothetical" new evolutionary scenario and configuration of subspecies. At the maximum

675 extent of Pleistocene glaciations, south of the ice sheet were wolves that had already evolved in

676 or colonized North America: *C. l. lycaon* in the east, *C. l. baileyi* in the southwest, and a

677 "southern group." The southern group corresponds to *C. l. nubilus* in Nowak's (1995) eventual

678 reclassification. North of the ice sheet were the ancestors of *C. l. arctos* in the east, and a

679 "northern group" isolated to the west in Alaska. This northern group corresponds to *C. l.*

680 *occidentalis* in Nowak's later revision.

681   Bogan and Mehlhop (1983) reported the results of principal component and discriminant

682 function analyses of 253 wolf skulls from Mexico and the southwest region of the United States,

683 including specimens from Kansas, Oklahoma, Texas, Colorado, New Mexico, and Arizona.

684   Samples were grouped by subspecies according to Goldman's (1944) classification. They

685   considered *Canis lupus mogollonensis* and *Canis lupus monstrabilis* to be synonyms of *C. l.*

686   *baileyi* based on broad morphological overlap of their skulls (Bogan and Mehlhop 1983, Figure 2

687   and Figure 3; Figure 2 is reproduced here as Figure 5). This effectively expanded the range of *C.*

688   *l. baileyi* north to central Arizona and New Mexico, and east into central Texas. They also

689   acknowledged that specimens previously referred to *C. l. mogollonensis* represented intergrades

690   between *C. l. bailey* and *C. l. youngi*, the subspecies then recognized for the southern Rocky

691   Mountains, and did not detect the abrupt break between *C. l. baileyi* and *C. l. mogollonensis*

692   noted by Goldman (1944). They recognized three subspecies in the area covered by their study:

693   *C. l. baileyi*, *C. l. youngi*, and in the Great Plains, *C. l. nubilus*.

694   Schmitz and Kolenosky (1985) reported clinal variation in *C. l. lycaon* (following

695   Goldman's [1944] delineation of the range of that subspecies) in Ontario based on discriminant

696   function analysis of skull and body characters. Canids were assigned to six groups for the

697   discriminant function analysis: boreal, Algonquin, and southern Ontario wolves; and Algonquin,

698   southeast Ontario, and southwest Ontario coyotes. From larger wolves in boreal regions in the

699   north, size declined to the smaller, Algonquin-type wolves in southern Ontario. They found that

700   the boreal wolves more resembled Minnesota wolves than Algonquin wolves in body characters,

701   but boreal more resembled Algonquin in skull characters. Their explanation was that the

702   resemblance between boreal and Minnesota wolves (which they viewed as derived from *C. l.*

703   *nubilus*) was owing to convergence based on similar prey size, and that resemblance between

704   boreal and Algonquin in skull characters was owing to taxonomic affinity and clinal variation

705   within *C. l. lycaon*.

706   Nowak (1995) performed discriminant function analyses using 580 male skulls grouped

707   by subspecies as delineated by Hall (1981), with *C. l. lycaon* further divided into three groups:

708   Minnesota, western Ontario, and Algonquin Provincial Park. Based on statistical distances (D^2

709   of Mahalanobis), Nowak (1995, Figure 20) revised the subspecies taxonomy of North American

710   *Canis lupus* in a manner generally consistent with the geographic groupings that he had proposed

711   in his1983 paper and reduced the number of subspecies from the 23 recognized by Goldman

712   (1944) to five. In this classification, most of the North American range of *C. lupus* is occupied

713   by *C. occidentalis* and *C. nubilus*, which corresponded to the respective northern and southern

714   groups identified earlier (Nowak 1983). The other three subspecies had smaller ranges on the

715   periphery of the North American range of *C. lupus*: *C. l. lycaon* in the east, *C. l. baileyi* in the

716   southwest, and *C. l. arctos* in the islands of the High Arctic.

717   Nowak's (1995) analysis included 105 individuals of *C. l. occidentalis* and 119

718   individuals of *C. l. nubilus*. An additional 46 specimens from subspecies that he included as

719   synonyms of *C. l. nubilus* were also included in the study. Statistical distances and discriminant

720   function plots (Nowak 1995, Figure 5, Figure 7; the latter is reproduced here as Figure 6)

721   separate *C. l. nubilus* and *C. l. occidentalis*. Polygons on the discriminant function plots

722   overlaped, but were mostly non-overlapping, with *C. l. occidentalis* being larger than *C. l.*

723   *nubilus*. *Canis lupus ligoni* (attributed by Nowak to *C. l. nubilus*) of coastal southeastern Alaska

724   was intermediate. Nowak (1995, p. 383) acknowledged that *C. l. ligoni* has probably been

725   affected by hybridization with *C. l. occidentalis*, but that it is partly isolated from *C. l.*

726   *occidentalis* to the east by "mountains, glaciers, and waterways," and has closer statistical

727   distance to *C. l. nubilus* to the south. He also found that samples from coastal British Columbia,

728   including Vancouver Island, were closer to *C. l. nubilus*.

729   Nowak (1995, p. 386, Figure 10) found that *C. l. hudsonicus* (a synonym of *C. l. nubilus*

730   in his classification), found west of Hudson Bay, was within the statistical limits of *C. l. nubilus*,

731   although it overlapped with the discriminant function polygon for *C. l. occidentalis*. He

732   suggested that Skeel and Carbyn's (1977) finding that *C. l. hudsonicus* was closer to *C. l.*

733   *occidentalis* from Wood Buffalo National Park may have involved inclusion of females, which

734   are smaller, in the male sample of the latter. He also suggested that habitat and prey preferences

735   may contribute to differences between *C. l. hudsonicus* and *C. l. occidentalis* and their

736   coexistence without merging or displacement, stating that *Canis lupus occidentalis* is mainly in

737   the taiga in this area, while *C. l. hudsonicus* is mainly in the less productive tundra.

738   The range delineated for *C. l. lycaon* included southeastern Ontario and southern Quebec.

739   Areas formerly considered by Goldman (1944) to be within the western range of *C. l. lycaon*

740   (Minnesota, the Upper Peninsula of Michigan, Wisconsin, and southwestern Ontario) were

741   included within the range of *C. l. nubilus*. Nowak (1995, Figures 5, 10) based this on the low

742   statistical distance between Minnesota wolves and historical *C. l. nubilus*, and on the occurrence

743   of individuals from western Ontario within the range of variation of his southern group, which is

744   equivalent to *C. l. nubilus*. *Canis lupus lycaon* from or near to Algonquin Provincial Park

745   overlapped to some degree with the western Ontario specimens, but were mostly outside the

746   polygon describing variation in *C. l. nubilus*.

747   Nowak (1995, Figures 4, 8) recognized *C. l. baileyi* as a subspecies, but did not adopt

748   Bogan and Mehlhop's (1983) inclusion of *C. l. mogollonensis* and *C. l. monstrabilis* as its

749   synonyms. These different interpretations are discussed later in the "Analysis and Discussion"

750   under *C. l. baileyi*.

751   The geographical ranges of *C. l. baileyi* and other subspecies of *C. lupus* may never have

752   had definite or stationary boundaries. Nowak (1995, p. 385) suggested that *C. l. baileyi*,

753   "regularly dispersed into the range of populations to the north and vice versa." He also

754   suggested that extirpation of more northern wolves facilitated the dispersal of *C. l. baileyi* from

755   Mexico to areas formerly occupied by other subspecies.

756   *Canis lupus arctos* partially overlaped both *C. l. nubilus* and *C. l. occidentalis* on

757   Nowak's (1995, Figure 9) discriminant function plot for some northern wolves. He recognized

758   *C. l. arctos* as a subspecies based on these results, along with the observation that their large

759   carnassial teeth were "the most consistent distinguishing character." He included *C. l. bernardi*

760   in *C. l. arctos* based on this character, and included *C. l. orion* based on examination of two

761   specimens and reported free movement (Dawes et al. 1986) of wolves between the northern

762   Greenland range of *C. l. orion* and the Ellesmere Island portion of the range of *C. l. arctos*.

763   The relatively small size of *C. l. baileyi* and *C. l. lycaon* invites their comparison with *C.*

764   *rufus*. Nowak's (1995) *C. rufus* sample was selected to exclude specimens that reflected

765   hybridization with coyotes, *Canis latrans*. These skulls were a series collected before 1930 in

766   southern Missouri, and another collected before 1940 from southeastern Texas to Florida. They

767   were compared with Algonquin Provincial Park *C. l. lycaon* from southeastern Canada and his

768   "southern group" of gray wolves, which is equivalent to *C. l. nubilus* and includes Minnesota

769   wolves collected after 1960. Nowak's (1995, Figure 11) discriminant function analysis of these

770   samples indicated that the areas of the discriminant function plot occupied by the Algonquin *C. l.*

771   *lycaon* and *C. rufus* individuals approach one another. He suggested the possibility that coyote

772   hybridization could have contributed to the closeness of *C. l. lycaon* to *C. rufus*. He observed

773   that there were too few specimens to evaluate whether *C. l. lycaon* and *C. rufus* intergraded. He

774   described the historical range of *C. rufus* as extending north to Pennsylvania and that of *C. l.*

775   *lycaon* as extending south from Quebec and Ontario "to an undetermined point in the eastern

776   United States."

777   Nowak's (1995, Figure 11) discriminant function analysis of *C. rufus* (n = 33) and *C.*

778   *baileyi* (n = 21) showed no overlap, although one *C. baileyi* individual was just outside the

779   polygon representing *C. rufus* individuals. Statistical distance values (Nowak 1995, Figure 12)

780   showed pronounced differences between 27 *C. l. monstrabilis* (a synonym of *C. l. baileyi*

781   according to Bogan and Mehlhop [1983]) and *C. rufus*. Nowak (1995, p. 389) observed that,

782   "there are no specimens to show that the gray wolf was sympatric with unmodified populations

783   of red wolf," although *C. lupus* was in the vicinity of areas of central Texas where *C. rufus* and

784   coyotes were hybridizing.

785   In recent papers, Nowak (2002, 2003, 2009) repeated his view that *C. l. lycaon* is a

786   subspecies of *C. lupus* and may be the result of hybridization that occurred when *C. rufus*

787   advanced north into Canada following the last Pleistocene glacial retreat and came into contact

788   with *C. lupus*, more specifically the subspecies *C. l. nubilus*. His discriminant function plots

789   showed that specimens attributed to *C. l. lycaon* (n = 10) are intermediate between western *C.*

790   *lupus* (n = 97) and *C. rufus* (n = 13) and slightly overlap *C. lupus* (Nowak 2002, figure 8; 2003,

791   figure 9.9).

792   In his review and discriminant function analysis of eastern *C. lupus* and *C. rufus*, Nowak

793   (2002) explored relationships between *C. l. lycaon* and *C. l. rufus*, as well as *C. lupus* from the

794   western Great Lakes region and some localities in the western United States. Historical *Canis*

795   *lupus lycaon* (n = 10) from southeastern Canada (Nowak 2002, Figure 8) overlaped only slightly

796   with a "western series" of *C. lupus* (ranging from Minnesota to Idaho and Arizona). A series

797   from the Upper Peninsula of Michigan had substantial overlap with both the eastern *C. l. lycaon*

798   and the western *C. lupus*. Based on the intermediacy of *C. l. lycaon* from southeastern Canada

799   between *C. l. nubilus* and *C. rufus*, Nowak (2002, 2003) suggested that *C. l. lycaon* may be the

800   result of hybridization as *C. l. nubilus* invaded from the west and encountered *C. rufus* invading

801   from the south following retreat of the terminal Pleistocene glaciations.

802   Nowak (2002, Figure 6) compared Minnesota *C. lupus* skulls (n = 23) taken after 1970

803   with five series of historical specimens from within the western range of *C. l. nubilus* (n = 78).

804   The Minnesota wolves overlapped strongly with the series from the northern Rocky Mountains

805   (of the United States), and less so with the southern Rocky Mountain series. There was slight

806   overlap with the Nebraska-Kansas-Oklahoma series, and none with the small sample from Texas.

807   To determine whether western *C. lupus* and *C. rufus* became more morphologically

808   similar where their ranges approach one another, Nowak (2002) performed a discriminant

809   function analysis that included seven skulls from western Texas. These individuals were from

810   within the range of *C. l. monstrabilis*, which is considered a synonym of *C. l. baileyi* by Bogan

811   and Mehlhop (1983), but is considered a synonym of *C. l. nubilus* by Nowak (1995). The

812   resulting discriminant function plot (Nowak 2002, Figure 6) indicated no morphometric

813   convergence between the Texas *C. lupus* and pre-1918 *C. rufus* (n = 6), which although based on

814   a limited sample of *C. lupus* specimens provided no evidence that interbreeding between these

815   species was occurring in western and central Texas.

816   Nowak (2003, Table 9.3; 2009, Figure 3) described the historical range of *C. l. lycaon* as

817   extending south to northern and western New York. Nowak (2003, p. 247) noted that a few

818   Pleistocene specimens indicate that *C. l. baileyi* once extended to Kansas and southern

819   California.

820   Nowak (2009) focused on the relationships among wolves of the Great Lakes area in a

821 discriminant function analysis of historical skulls intended to portray patterns of variation before

822 wolves were exterminated from much of the area. In the initial comparison of series representing

823 western *C. l. nubilus* (collected before 1930, n = 27), northern Minnesota wolves (1970-1975, n

824 = 23), and *C. l. lycaon* from Algonquin Provincial Park, Ontario (1964-1965, n = 20), there was

825 no overlap between *C. l. lycaon* and either the northern Minnesota or the western *C. l. nubilus*.

826 Most Minnesota series, however, overlapped the polygon for *C. l. nubilus* (Nowak 2009, Figure

827 1). Nowak (2009, Figure 15.2) then compared these relationships with two series that are

828 geographically intermediate between Minnesota and Algonquin Provincial Park: Upper

829 Peninsula of Michigan (collected prior to 1966), and a series collected between the Upper

830 Peninsula and Algonquin Provincial Park. Each of these two series overlaped both Minnesota

831 and Algonquin on the discriminant function plots, thus bridging the morphological gap between

832 them.

833   Mulders (1997) used principal components and discriminant function analyses to study

834 skulls of 525 *Canis lupus* from Canada. He found the wolves of the Canadian Arctic Islands (*C.*

835 *l. arctos* and *C. l. bernardi*) to be distinct from mainland wolves, but not from each other. He

836 interpreted his findings as supporting recognition of the subspecies *C. l. arctos* and Nowak's

837 (1995) treatment of *C. l. bernardi* as its junior synonym. He found support for *C. l. occidentalis*

838 and *C. l. nubilus*, but with boundaries different from those proposed by Nowak (1995). He

839 characterized *C. l. occidentalis* as "mainland tundra wolves," with a range including Yukon,

840 Northwest Territories, Baffin Island, and portions of Manitoba and western Ontario in the

841 vicinity of Hudson Bay. He characterized *C. l. nubilus* as "central boreal wolves," with a range

842   south of *C. l. occidentalis*, including eastern British Columbia, Alberta, and nearly all of

843   Saskatchewan; these areas were included within the range of *C. l. occidentalis* by Nowak (1995).

844   Mech and Paul (2008) accepted the recognition of *C. lycaon* as a species separate from *C.*

845   *lupus*. Based on their analysis of body mass of 950 female and 1006 male adult wolves from

846   across northern Minnesota, they describe an increasing trend in body mass from east to west for

847   both sexes. They concluded that this trend supports the view that the two species meet and

848   hybridize in northern Minnesota. *Canis lupus* in this study would represent *Canis lupus nubilus*

849   according to Nowak's (1995) distribution map.

850

851   *Summary of studies on morphology*. Studies with comparable geographic coverage agree in

852   indicating smaller wolves in the Great Lakes region (eastern wolf), with size increasing to the

853   north and west of that region. The study of Lawrence and Bossert (1967) is not comparable

854   because the influence of size was reduced or eliminated by the selection of specimens and the

855   use of ratios rather than direct measurements of skull characters.

856   Studies involving *Canis lupus bailey* came to different conclusions as to its northern

857   boundary, with Bogan and Mehlhop (1983) favoring a more northerly boundary than Nowak

858   (1995).

859

860   *Autosomal genetic markers*. Broad patterns of variation in North American *Canis* were

861   investigated (Roy et al. 1994, 1996) using microsatellite DNA. Samples of *C. lupus* from

862   Minnesota and southern Quebec as well as red wolves (*Canis rufus*) were intermediate between

863   two large multi-dimensional scaling clusters (Roy et al. 1996, Figure 3, which is reproduced here

864   as Figure 7) representing five populations each of *C. lupus* and coyotes (*C. latrans*). The red

865 wolf samples (n = 40, Roy et al. 1994) were from the captive breeding program (derived from

866 red wolves captured in southeastern Texas and southwestern Louisiana [Roy et al. 1994]) and

867 pre-1940 individuals (n = 16, Roy et al. 1996) from Texas, Oklahoma, Arkansas and Missouri.

868 Roy et al. (1994, 1996) attributed the intermediate placement of these red wolves as well as

869 Minnesota and southern Quebec *C. lupus* to extensive hybridization between *C. lupus* and

870 coyotes. The possibility of an original evolutionary affinity between *C. rufus* and wolves from

871 southern Quebec and Minnesota was not discussed. The *C. lupus* of northern Quebec (n = 20,

872 Roy et al 1994) were closer to western *C. lupus* from Vancouver Island (n = 20), Alberta (n =

873 20), and Kenai Peninsula, Alaska (n = 19). A neighbor-joining analysis (Roy et al. 1994, Figure

874 7) of Nei's (1978) genetic distance found northern Quebec wolves to be intermediate between

875 southern Quebec and Minnesota wolves and western *C. lupus* from Vancouver Island, Alberta,

876 Northwest Territories and Alaska. Red wolves were intermediate to southern Quebec and

877 Minnesota wolves and coyotes.

878 García-Moreno et al. (1996) compared microsatellite DNA variation in *C. l. baileyi* from

879 the captive breeding program with 42 dogs and the gray wolf, coyote and red wolf data presented

880 by Roy et al. (1994). A multi-dimensional scaling plot of the microsatellite data (García-Moreno

881 et al. 1996, Figure 4), showed pronounced separation of *C. l. baileyi* from all the other canids,

882 including other *C. lupus*, although the authors acknowledged that the effects of small founder

883 size and genetic drift in the captive *C. l. baileyi* population may have contributed to their genetic

884 distinctiveness (Paetkau et al. 1997). Red wolves were not included in the Minnesota/Quebec

885 wolf cluster in the MDS plot, but appeared within the confidence ellipse of coyotes (García-

886 Moreno et al. 1996, Figure 4).

887   A neighbor-joining tree (García-Moreno et al. 1996, Figure 5) based on Nei's (1978)

888   genetic distance displayed the captive *C. l. baileyi* lineages close together on a well-supported

889   branch distinct from other *C. lupus*. The same neighbor-joining tree placed the *C. lupus* samples

890   from Minnesota basal to the clade comprised of *C. l. baileyi*, western gray wolves and domestic

891   dogs while the southern Quebec wolves were basal to the coyote – red wolf clade. Regardless,

892   both the Minnesota and southern Quebec populations were described as "hybridizing gray

893   wolves."

894   Several detailed studies have used autosomal microsatellite DNA to characterize the

895   population genetics of wolf recovery in the Northern Rocky Mountains of the United States

896   (Forbes and Boyd 1996, 1997; vonHoldt et al. 2008, 2010). The Montana population is

897   descended from wolves that naturally dispersed from southern Alberta and British Columbia,

898   whereas the Idaho and Yellowstone National Park populations were founded with re-

899   introductions from central Alberta and northern British Columbia. These populations represent

900   the single taxon, *Canis lupus occidentalis* in Nowak's (1995) classification. These studies

901   provide fascinating illuminations of pack structure, reproductive behavior, and migration but are

902   not informative on the taxonomic questions that are the subjects of this paper and will not be

903   further considered.

904   In a study concentrating on the evolutionary relationships of the wolves of eastern

905   Canada, Wilson et al. (2000) reported microsatellite variation at eight of the loci used by Roy et

906   al. (1994) in comparisons of *Canis* from the vicinity of Algonquin Provincial Park, Ontario

907   (putatively *C. lycaon*), suspected hybridizing (wolf/coyote) wolves from southern Quebec and

908   Minnesota, *C. rufus* from the red wolf captive breeding program, and *C. lupus* from Northern

909   Ontario, Alberta, and the Northwest Territories. This study also described mitochondrial DNA

910   (mtDNA) control region sequence variation that will be discussed later. Issues of particular

911   interest were the relationship of *C. lycaon* to *C. rufus* and how hybridization with coyotes may

912   have contributed to the genetic similarities observed between them.

913   Neighbor-joining trees based on Nei's genetic distance (1972) (Wilson et al. 2000,

914   Figures 1 and 2) grouped *C. rufus* with wolves from Algonquin Provincial Park, southern

915   Quebec, and Minnesota, but separate from both coyotes and western *C. lupus*. They concluded

916   that the similarity between eastern Canadian wolves and *C. rufus* was not due to shared

917   introgression from coyotes, because alleles found in the coyote populations were either absent or

918   found at low frequency in *C. rufus* (Wilson et al. 2000, Table 1). At these loci captive *C. rufus*

919   were more similar to Algonquin wolves than to coyotes from Texas, an expected source of

920   introgression into the founders of the captive red wolf population.

921   Individual assignment tests also indicated Algonquin Provincial Park wolves and *C. rufus*

922   were distinct (probability of identity measure) or nearly distinct (individual index) from Texas

923   coyotes (Wilson et al. 2000, Figure 3). These analyses also supported the mutual distance of

924   Algonquin wolves and *C. rufus* from western *C. lupus* (Wilson et al 2000, Figure 4).

925   Hedrick et al. (2000) compared major histocompatibility complex variation (MHC)

926   among Mexican wolves and red wolves from the respective recovery programs with western

927   coyotes. They found that Mexican wolves did not share alleles with red wolves or California

928   coyotes, but one allele in the Aragon lineage of Mexican wolves was shared with other gray

929   wolves (Hedrick et al. 2000). Red wolves shared one of their alleles with gray wolves. Further

930   study of MHC variation with additional coyote samples (Hedrick et al. 2002) found that three of

931   the four red wolf haplotypes were shared with coyotes, consistent with their recent history prior

932   to the establishment of the captive population (Wayne and Jenks 1991).

933   Carmichael et al. (2001) studied microsatellite variation in 491 *C. lupus* from nine

934   locations in the Northwest Territories and Yukon of Canada. Analysis of genetic distance using

935   F_{ST}, the fixation index of Wright (1951), and assignment tests all indicated restricted gene flow

936   between wolves on different sides of the Mackenzie River ($F_{ST} = 0.04$) and little differentiation

937   among wolves on the same side (F_{ST} ranged 0.01 to 0.02). Rather than topological isolation, the

938   authors associated this barrier with predator-prey specialization to different caribou herds with

939   seasonal migratory patterns that were exclusive to either side of the river. Genetic distances

940   observed between Arctic Island (*C. l. arctos* in Nowak 1995) and mainland (*C. l. occidentalis* in

941   Nowak 1995) wolves (F_{ST} ranged 0.09 to 0.19) were twice that observed among mainland

942   wolves (F_{ST} ranged 0.01 to 0.1) (Carmichael et al. 2001, Table 2).

943   In a study designed to explore pack composition among wolves in Algonquin Provincial

944   Park, Grewal et al. (2004) assessed parent-offspring relationships from mtDNA control region

945   sequence as well as Y-chromosome and autosomal microsatellite variation. Wolves at proximal

946   locations in Frontenac Axis (n = 74) and Magnetawan region (n = 26), as well as Northeast

947   Ontario (n = 33), Abitibi-Temiscamingue region (n = 13), and La Verendrye Reserve in

948   southeast Quebec (n = 13) were also assessed. The data from the mtDNA sequence and Y-

949   chromosome microsatellite portions of this study will be discussed in following sections.

950   In addition to breeding adults and offspring, most packs were found to have at least one

951   additional, non-breeding and unrelated adult. STRUCTURE analysis identified five immigrants

952   into the Algonquin Provincial Park: three from Frontenac Axis, one from Magnetawan region,

953   and one from north of the park. Wolves from the Frontenac Axis and Magnetawan region

954   locations exhibited significant introgression from coyote, whereas the genotypes of wolves from

955   north of the park (within the range of *C. l. nubilus* in Nowak 1995) predominantly exhibited *C.*

956 *lupus* alleles. Algonquin Provincial Park wolves (within the range of *C. l. lycaon* in Nowak 1995

957 and *C. lycaon* in Wilson et al. 2000) were differentiated from proximal populations (F_{ST} ranged

958 0.024 to 0.055), but were approximately twice as divergent from wolves from the more distant

959 Abitibi-Temiscamingue region (F_{ST} = 0.089), La Verendrye Reserve (F_{ST} = 0.091) and

960 Northeastern Ontario (F_{ST} = 0.076) localities.

961   Weckworth et al. (2005) compared microsatellite variation among wolf populations from

962 coastal southeast Alaska (n = 101; *C. l. nubilus* in Nowak 1995) and coastal south-central

963 Alaska, interior Alaska, Northwest Territories and British Columbia (n = 120; *C. l. occidentalis*

964 in Nowak 1995) and found that the coastal and continental groups were distinguished by

965 significant differences in allele frequencies. Mean distance between coastal and continental

966 wolves (average F_{ST} = 0.16) was nearly twice that of the mean distance within groups (average

967 F_{ST} = 0.09). However, wolves from coastal south-central Alaska and coastal southeast Alaska

968 were similarly distinguished (average F_{ST} = 0.16). The authors attributed the observed genetic

969 divergence to the waterways, high mountains, and glaciers barring the dispersal of wolves

970 (Weckworth et al. 2005, Figure 2). They also hypothesized that southeast Alaska was colonized

971 from the south with the retreat of the last Pleistocene glaciation whereas interior Alaska had been

972 colonized by wolves from the Beringian refugium to the north (Nowak 1983).

973   Musiani et al. (2007) compared microsatellite DNA variation in gray wolves from seven

974 localities in tundra/taiga habitat in Northwest Territories, Canada (n = 337) and four localities in

975 boreal forest habitat in Northwest Territories and northern Alberta (n = 67) to examine the

976 effects of habitat specializations on population structure. GENELAND analysis combined the

977 wolves from tundra/taiga and boreal forest localities into respective populations, and found

978 significant genetic differentiation (F_{ST} = 0.03) between the two. As genetic and geographic

979 distances were not significantly correlated, and topological barriers were not in evidence, the

980 authors concluded that prey and habitat specialization had promoted the genetic differentiation.

981 The autosomal and Y-chromosome microsatellite data obtained in this study are discussed at

982 length elsewhere.

983 Carmichael et al. (2007, 2008) explored geographic variation in wolves inhabiting the

984 North American Arctic. Carmichael et al. (2007) focused on the structuring of mainland

985 populations, Carmichael et al. (2008) on Canadian Arctic Islands wolves. The wolves genotyped

986 in these studies included individuals from the Canadian Arctic Islands (n = 342), southeast

987 Alaska coastal islands (n = 35) and mainland locations (n = 1648) that were attributable to three

988 different subspecies following Nowak's (1995) reclassification and general subspecies

989 boundaries: *C. l. occidentalis*, *C. l. nubilus*, and *C. l. arctos*. Ten populations (Carmichael et al.

990 2007, Figure 2(b)) were identified through the combined results of STRUCTURE and

991 GENELAND analyses. Carmichael et al. (2007) explained this population divergence in terms

992 of reduced dispersal due to topographic, habitat selection, and prey preference barriers. They

993 noted that the geospatial distribution of the recognized populations did not correspond to the

994 morphological subspecies boundaries in Nowak (1983, 1995), but reflected contemporary factors

995 affecting gene flow.

996 Sampling areas attributable to *C. l. occidentalis* were generally grouped together in a

997 neighbor-joining tree of genetic distance (Carmichael et al. 2008, Figure 3B). However, the

998 sample group from southeast Alaska coastal islands (*C. l. nubilus*, after Nowak 1995) was more

999 similar to proximal interior populations in Alaska, Yukon, Northwest Territories and British

1000 Columbia (*C. l. occidentalis*, after Nowak 1995) than to the cluster of other *C. l. nubilus*

1001 sampling areas from Nunavut (Qamianirjuaq and Bathurst) and Atlantic Canada. The Manitoba

1002   and British Columbia samples that straddled the eastern and western boundaries between *C. l.*

1003   *occidentalis* and *C. l. nubilus* were both included within the *C. l. occidentalis* portion of the tree.

1004   Of the *C. l. nubilus* sampling areas, Baffin Island and an adjacent mainland area occurred

1005   together on a well-supported branch, but the wolves of the Atlantic sampling area (including

1006   Labrador, New Foundland, New Brunswick, and Nova Scotia) were as divergent from the Baffin

1007   NE Mainland population as they were from the Victoria/Banks/High Arctic Islands wolves

1008   (Carmichael et al 2008, Table 3).

1009   Consistent with recent re-population from the south rather than having occupied a

1010   separate glacial refuge north of the ice sheet as proposed by Nowak (1983), Canadian Arctic

1011   Island wolves exhibited few unique alleles. The lack of isolation of island and mainland wolf

1012   populations was further supported by the observation of high gene flow between mainland and

1013   island wolf populations (Carmichael et al 2008, Table 2) and annual over-ice, island-mainland

1014   migrations of island wolves (Carmichael et al. 2001) and their caribou prey (Carmichael et al.

1015   2008, Figure 4). Although genetic distance between island and mainland populations was

1016   generally much higher (D_s ranged 0.08 to 0.63) than among mainland populations (D_s ranged

1017   0.01 to 0.30) (Carmichael et al. 2008, Table 3), the effects of small founder size and genetic drift

1018   may have contributed to the genetic distinctiveness of Arctic Islands wolves (Paetkau et al 1997).

1019   For example, the High Arctic and Victoria Island populations as well as the southeast Alaska

1020   coastal islands populations exhibited evidence of inbreeding (F_{IS} ranged 0.18 to 0.63). As a

1021   result, the authors recommended that conclusions about, "the taxonomic validity of *C. l. arctos*

1022   should be deferred," until data are available from mtDNA and Y-chromosome sequences

1023   (Carmichael et al. 2008, p. 886).

1024   Wheeldon and White (2009) successfully genotyped three historical wolf specimens from

1025   Minnesota and Wisconsin (1899-1908) at six microsatellite loci and characterized them in a

1026   STRUCTURE analysis along with wolf and coyote samples from throughout Canada. The

1027   microsatellite profiles of both modern and historical western Great Lakes wolves exhibited

1028   similar admixture proportions that were attributed to *C. lupus*-*C. lycaon* hybridization. These

1029   results suggested that *C. lupus* and *C. lycaon* were sympatric and interbreeding prior to their

1030   extirpation from the region in the early twentieth century.

1031   Koblmüller et al. (2009a) used autosomal microsatellite, Y-chromosome microsatellite,

1032   and mtDNA sequence analysis to compare modern Great Lakes wolves with eastern (n = 49) and

1033   western (n = 78) coyotes, western *C. lupus* (n = 58), and historical Great Lakes wolves (collected

1034   prior to the modern establishment of coyotes in the region). The modern Great Lakes wolf

1035   sample consisted of some (n = 64) of the same Minnesota animals analyzed by Lehman et al.

1036   (1991) and Roy et al. (1994), recovered Wisconsin (n = 16) and Upper-Peninsula Michigan

1037   wolves (n = 63), as well as wolves from Ontario (n = 51) and Quebec (n = 9). The historical

1038   sample (n = 19) included wolves from Minnesota, Wisconsin, Michigan, Ontario, Quebec, and

1039   New York. The Y-chromosome and mtDNA data are discussed separately.

1040   STRUCTURE analysis of the Northwest Territories and Great Lakes wolf samples from

1041   Minnesota, Wisconsin, and Michigan revealed little evidence of admixture, whereas, the authors

1042   interpreted the significant admixture observed in wolves from Ontario and Quebec as the result

1043   of recurrent hybridization between gray wolves and Great Lakes wolves and between Great

1044   Lakes wolves and coyotes. Genetic divergence was reported in this paper using the notation Θ_{ST},

1045   which is simply Θ, the coancestry parameter, in the original notation of Weir and Cockerham

1046   (1984). Little difference was detected between the modern recovered population of Great Lakes

1047   wolves and the historical sample (Θ = 0.036). Great Lakes wolves were equally divergent from

1048   eastern (Θ = 0.142) and western (Θ = 0.133) coyote, but less so from western *C. lupus* (Θ =

1049   0.078). The authors did not conclude that Great Lakes wolves constituted a separate species, but

1050   rather a unique population or ecotype of *C. lupus*.

1051   Wolf-dog hybridization in the wolf population of Vancouver Island was the subject of

1052   study by Muñoz-Fuentes et al. (2009b) using microsatellites and mtDNA control region

1053   sequence. Wolves were thought to have been extirpated from the island between 1950 and 1970,

1054   after which they re-colonized from the adjacent mainland of British Columbia. In contrast to the

1055   mtDNA data, the microsatellite data did not reveal evidence of dog introgression. The authors

1056   concluded that these results were consistent with a single hybridization event occurring early in

1057   the re-establishment of wolves on the island.

1058   Wilson et al. (2009) explained the genetic relationships of three "races" of wolves living

1059   in Ontario, Canada: the larger Ontario and smaller Algonquin types of the eastern timber wolf

1060   (*C. l. lycaon*, Kolenosky and Standfield 1975) and the Tweed wolf that is thought to be a wolf-

1061   coyote hybrid. The authors assessed the relationships of wolves from across Ontario for

1062   evidence of hybrid admixture from autosomal microsatellite variation. The study included

1063   wolves from both southern regions of Ontario: Frontenac Axis (n = 74), Magnetawan region (n =

1064   26) and Algonquin Provincial Park (n = 92); and northern regions: northeast Ontario (n = 33),

1065   northwest Ontario (n = 30) and Pukaskwa National Park (n = 13).

1066   STRUCTURE analysis segregated the sample into three populations (Wilson et al. 2009,

1067   Figure 3): wolves from the northern regions, which also exhibited *C. lupus* mtDNA (Old World

1068   type, Wilson et al. 2009, Table 2) at high frequency (24-85%), eastern wolves from Algonquin

1069   Provincial Park (3% *C. lupus* mtDNA), and Tweed wolves from the Magnetawan region and

1070   Frontenac Axis in the south (100% *C. latrans*/*C. lycaon* mtDNA, New World type, Wilson et al.

1071   2009, Table 2). The microsatellite genotypes of Frontenac Axis and Magnetawan region wolves

1072   were significantly introgressed with coyote alleles (> 50% of population), whereas those from

1073   north of the park (within the range of *C. l. nubilus* in Nowak 1995) exhibited genotypes of

1074   predominantly gray wolf alleles (> 70% of population) (Wilson et al. 2009, Figure 4).

1075   Algonquin Provincial Park wolves (within the range of *C. l. lycaon* in Nowak 1995 and *C.*

1076   *lycaon* in Wilson et al. 2000) were differentiated from southern Magnetawan region and

1077   Frontenac Axis populations (F_{ST} ranged 0.022 to 0.055, respectively), but were twice as

1078   divergent from northern region wolves (F_{ST} ranged 0.071 to 0.117) (Wilson et al. 2009, Table 5).

1079   The authors concluded that eastern wolves of the smaller Algonquin type (Kolenosky and

1080   Standfield 1975) are *C. lycaon*, eastern wolves of the larger Ontario type (Kolenosky and

1081   Standfield 1975) are *C. lycaon*-*C. lupus* hybrids, and Tweed wolves are *C. lycaon*-coyote

1082   hybrids.

1083   Fain et al. (2010) tested the influence of hybridization on wolf recovery in the western

1084   Great Lakes states. Microsatellite DNA variation was compared in the recovered Great Lakes

1085   states wolf population from Minnesota, Wisconsin and Upper Peninsula Michigan (n = 112);

1086   western gray wolves from Alaska, British Columbia and Alberta (n = 103); Wisconsin coyotes (n

1087   = 36) and domestic dogs (n = 39).

1088   STRUCTURE analysis clearly segregated Great Lakes states wolves, western gray

1089   wolves, coyotes and dogs (Fain et al. 2010, Figure 6); however, the Great Lakes sample included

1090   *C. lupus* x *C. lycaon* hybrids (25%). Divergence between Great Lakes wolves (within the range

1091   of *C. l. nubilus* in Nowak 1995) and western *C. l. occidentalis* (Nowak 1995) was high (F_{ST} =

1092   0.125), and Great Lakes wolves were equally divergent from domestic dogs (F_{ST} = 0.123).

1093   Moreover, the analysis showed that western Great Lakes wolves and sympatric coyotes were also

1094   highly differentiated (F_{ST} = 0.159). The authors concluded that this result was inconsistent with

1095   recent hybridization. The mtDNA and Y-chromosome data also obtained in this study are

1096   discussed separately.

1097   Rutledge et al. (2010b) compared microsatellite DNA variation in gray wolves from

1098   northeast Ontario (n = 51), eastern wolves from Algonquin Provincial Park (n = 128) and

1099   coyotes from Frontenac Axis (n = 38) in eastern Ontario in order to examine the effects of

1100   hybridization on population structure. GENELAND analysis segregated the three localities

1101   (separated by 700km) into genetically differentiated populations (F_{ST} ranged 0.052 to 0.120), but

1102   there was evidence of admixture. Algonquin Provincial Park wolves were admixed with both

1103   northeast Ontario (n = 8, 6%) and Frontenac Axis (n = 14, 11%) wolves, and over 15% of

1104   northeast Ontario and Frontenac Axis wolves were admixed with Algonquin Provincial Park

1105   wolves. Principle components analysis of individual autosomal microsatellite genotypes placed

1106   Algonquin Provincial Park wolves closest to Frontenac Axis wolves and revealed a south-north

1107   cline in allele frequencies. These results were considered supportive of the hypothesis that

1108   eastern wolves in Algonquin Provincial Park represent a conduit of gene flow between gray

1109   wolves to the north and coyotes to the south. In addition, mtDNA and Y-chromosome haplotype

1110   frequency distributions were consistent with the hypothesis that introgression was gender-biased

1111   with females of the smaller species mating males of the larger species. Conspecific pairings at

1112   Algonquin Provincial Park were more common than predicted by random mating. The mtDNA

1113   and Y-chromosome data obtained in this study are discussed further in following sections.

1114   Wheeldon et al. (2010) evaluated the species distinction of the wolves from the western

1115   Great Lakes region. The authors analyzed species-specific mtDNA and Y-chromosome

1116   sequence haplotypes in addition to autosomal microsatellite variation. The Y-chromosome and

1117   mtDNA sequence haplotype data are discussed elsewhere. The sample included 410 wolves and

1118   coyotes from the western Great Lakes states (Minnesota, Michigan, and Wisconsin) and western

1119   Ontario. Wolves and coyotes were distinguished as separate populations in both STRUCTURE

1120   and factorial correspondence analysis with little indication of hybridization. Y-chromosome and

1121   mtDNA haplotypes of both eastern and gray wolves were found in the wolf sample. The lack of

1122   subdivision of the wolf sample in the STRUCTURE analysis was cited as evidence that the

1123   wolves of the western Great Lakes states and western Ontario are members of a single

1124   interbreeding population.

1125

1126   *Summary of studies of autosomal genetic markers.* Studies of autosomal microsatellite DNA

1127   generally distinguish groups representing western gray wolves, eastern wolves (alternatively

1128   referred to as Great Lakes wolves in some studies), red wolves, Mexican wolves, and coyotes.

1129   Most studies in the western Great Lakes region, found the wolves to comprise an

1130   admixed population. An exception is the study of Koblmüller et al. (2009a), which claimed little

1131   admixture in the western Great Lakes region. There is disagreement on the source of the

1132   admixture: those who claim it represents gray wolf-coyote interbreeding (Roy et al. 1994, 1996;

1133   García-Moreno et al. 1996), others gray wolf-eastern wolf interbreeding (Wilson et al. 2000,

1134   Wheeldon and White 2009, Wilson et al. 2009, Fain et al. 2010, Wheeldon et al. 2010).

1135   Autosomal microsatellite DNA data were interpreted by Carmichael et al. (2007, 2008) as

1136   failing to support Nowak's interpretation, based on his morphometric analysis, of long isolation

1137   and subspecific validity of the arctic wolf, *Canis lupus arctos*.

1138

1139 *Single nucleotide polymorphisms (SNPs).* vonHoldt et al. (2011) report the results of an analysis

1140 of 48,036 single nucleotide polymorphism (SNP) markers in worldwide samples of dogs and

1141 wild canids. Samples of wild canids from North American include 57 coyotes, 12 red wolves, 19

1142 Great Lakes wolves, and 70 gray wolves. In a principal components analysis (vonHoldt et al.

1143 2011, Figure 3), the first component (accounting for 10% of the total variance) separated

1144 domestic dogs from the wild canids, and the second principal component (accounting for 1.7% of

1145 the variance) separated coyote and red wolf from other North American wolves. A successive

1146 series of STRUCTURE analyses first distinguished dogs from wild North American canids (K

1147 =2), coyote and red wolf from other wolves (K =3), Mexican wolf from other gray wolves (K =

1148 6), red wolf from coyotes (K = 9), and Great Lakes wolves from gray wolves (K = 10). A

1149 SABER analysis was performed on Great Lakes wolves (including two individuals from

1150 Algonquin Provincial Park) and red wolves using western gray wolves, dogs, and western

1151 coyotes as ancestral reference populations.

1152   Conclusions by vonHoldt et al. (2011) include: red wolf is an admixed variety derived

1153 from coyote; Great Lakes wolf is an admixed variety derived from gray wolves; Great Lakes

1154 wolf is genetically distinct from other gray wolves; Mexican wolf is a distinct form of gray wolf.

1155 Within gray wolves, geographic variation in SNP composition is attributed to geographic

1156 variation in ecological conditions rather than to taxonomic distinctions.

1157

1158 *Mitochondrial DNA.* Lehman et al. (1991) used restriction analysis of the entire mtDNA genome

1159 to describe haplotype variation in wolves and coyotes in areas of sympatry as well as isolated

1160 regions of allopatry in order to assess the occurrence of inter-species hybridization. The sample

1161 included wolves from Minnesota, Isle Royale in Lake Superior, western Ontario, Algonquin

1162   Provincial Park, Quebec, as well as selected western localities extending to Nome, Alaska. A

1163   maximum parsimony tree (Lehman et al. 1991, Figure 3) separated western *Canis lupus* from

1164   coyotes and wolves from the Great Lakes region. Although some Great Lakes wolves exhibited

1165   *C. lupus* haplotypes, most shared haplotypes with coyotes, or had "coyote-type" haplotypes (i.e.,

1166   phylogenetically similar but not observed in coyotes). All wolves sampled from southern

1167   Quebec and southeastern Ontario exhibited either coyote or coyote-type haplotypes. Lehman et

1168   al. (1991) concluded that Great Lakes wolves have a history of repeated hybridization with

1169   coyotes with the consequent introgression of coyote mtDNA haplotypes. They suggested that

1170   "Boreal type" (in the sense of Kolenosky and Standfield [1975]) and *C. l. lycaon* from north of

1171   the portion of their range also occupied by coyotes may represent "pure wolf lines" while the

1172   "Algonquin type" wolves to the south have been subject to coyote introgression. Because the

1173   most common coyote-type haplotypes found in Minnesota wolves were not found in coyotes, the

1174   authors suggested that hybridization involving those wolves occurred "in the distant past." They

1175   also stated that, as both *C. lupus* and coyote-type haplotypes occurred in individuals within the

1176   same packs, the Great Lakes region may contain a complex mix of *C. lupus*, coyotes, and their

1177   hybrids.

1178   Wayne and Jenks (1991) evaluated the genetic integrity of the source population from

1179   which the founders of the captive red wolf breeding program were selected. Seventy-seven

1180   canids were captured in southeastern Texas and southwest Louisiana in the 1974-1976 effort to

1181   rescue the last remaining wild *C. rufus*, and included the four matrilines used to found the red

1182   wolf captive breeding program (USFWS 1990). These animals were characterized

1183   morphologically as coyotes (58%), *C. rufus*-coyote hybrids (31%), and *C. rufus* (11%). Genetic

1184   characterization of these same animals with whole mtDNA genome restriction analysis found

1185   that they exhibited either coyote (84%) or gray wolf (16%) mtDNA and that the morphological

1186   and genetic classifications often did not correspond. Moreover, seven individuals (9% of the

1187   sample) exhibited a mtDNA restriction type previously observed only in Mexican wolves (*C. l.*

1188   *baileyi*), and parsimony analysis placed the mtDNA restriction type observed in captive red

1189   wolves within the phylogenetic clade composed exclusively of coyote types. The captive red

1190   wolf type was indistinguishable from a haplotype (i.e., C32) found in coyotes from Louisiana. In

1191   another part of this study, a portion of the mtDNA cytochrome *b* gene sequence was determined

1192   from historical (1905-1930) museum skins identified as *C. rufus* from Texas (n = 1), Louisiana

1193   (n = 1), Arkansas (n = 2), Oklahoma (n = 1), and Missouri (n = 1). Sequences were compared to

1194   similarly characterized gray wolf (n = 2), Mexican wolf (n = 1), coyote (n = 6), dog (n = 1), and

1195   golden jackal (n = 1) reference samples. The *C. l. baileyi* haplotype differed by only a single

1196   substitution from sequences of three historical red wolf specimens from Louisiana, Oklahoma,

1197   and Texas, which had a haplotype identical to a *C. lupus* individual from Minnesota. The *C. l.*

1198   *baileyi* haplotype was 10 to 19 nucleotide substitutions different from those of *C. rufus* from

1199   Arkansas, Missouri, and the captive population.

1200   Wayne et al. (1992) used whole mtDNA genome restriction analysis to study variation in

1201   *Canis lupus*, mostly from North America (n = 204), but included Eurasian samples for

1202   comparison (n = 35). North American wolves exhibited five haplotypes, Eurasian wolves seven,

1203   and none were shared. Subspecies assignments were not identified, but the distributions of wolf

1204   haplotypes W1 through W4 among North American wolves may have a bearing on the standing

1205   and relationships of the subspecies *C. l. nubilus* and *C. l. occidentalis*. The W3 haplotype was

1206   the most common and widespread, from Alaska to Montana, but not Manitoba, which all (n = 4)

1207   had the unique W2 haplotype. Haplotype W1 was found from the Northwest Territories to

1208 Montana (and later also found in Minnesota and central Ontario [Wayne et al. 1995]), and W4

1209 was found in scattered locations from Alaska to Montana. These haplotypes all differed by one

1210 or two restrictions sites (about 0.1 to 0.2% sequence divergence). The fifth haplotype (W14) was

1211 found only in the *C. l. baileyi* samples from the Mexican wolf captive breeding program. This

1212 haplotype was most closely associated with Eurasian *C. lupus* in the phylogenetic analysis

1213 (Wayne et al. 1992, Figure 2). Altogether, eastern wolves (n = 106) from Manitoba, Minnesota,

1214 Michigan, Ontario and Quebec exhibited seven "coyote-derived" haplotypes.

1215   Roy et al. (1996) tested the hypothesis that red wolves are evolutionarily ancestral to both

1216 gray wolves and coyotes but had undergone a recent interval of interbreeding with coyotes at the

1217 time of their near extermination in the wild. A portion of the mtDNA cytochrome *b* gene was

1218 sequenced from historical (1919-1943) museum skins of *C. rufus* (n = 11) and compared to those

1219 described by Wayne & Jenks (1991) from additional historical *C. rufus* (n = 6), captive *C. rufus*

1220 (n = 1), *C. lupus* from Alaska (n = 1), *C. lupus* from Minnesota (n = 1), *C. l. baileyi* (n = 1), *C.*

1221 *latrans* (n = 5), and *C. aureus* (n = 1). The observed haplotypes comprised two major clades,

1222 one including all coyotes, the other gray wolves. Most *C. rufus* were placed in the "coyote

1223 clade" (n = 8), but others from Missouri and Oklahoma (n = 3) were included in the wolf clade.

1224 Only haplotype CruOK3 was common to the different sets of historical red wolf samples.

1225   Sequence divergences were consistent within species: *C. rufus* (0.4 to 0.9%), *C. latrans*

1226 (0.4 to 1.7%) and *C. lupus* (0.4 to 1.3%). Sequence divergence between *C. lupus* and *C. latrans*

1227 was about four-fold greater (3.2-5.6%) and *C. lupus* were over 3.2% divergent from *C. rufus*

1228 from Arkansas, Oklahoma, and Texas, but less than 2.1% divergent from *C. rufus* from Missouri

1229 and Oklahoma. The authors concluded that introgression of *C. lupus* into *C. rufus* had occurred

1230 in Oklahoma and Missouri but not in Arkansas and Texas. The divergence observed between the

1231 16 historical *C. rufus* sequences and gray wolf and coyote references was consistent with the

1232 amount of within species variation found in gray wolves and coyotes. The results of the

1233 microsatellite analysis performed in this study are discussed in the preceding section.

1234   Vilà et al. (1999) included data from North American *C. lupus* (n = 24) in a broad-scale

1235 phylogeographic study of mtDNA control region sequence variation in wolves (Vilà et al. 1999,

1236 Figure 1, which is reproduced here as Figure 8). The phylogenetic tree that resulted from a

1237 neighbor-joining analysis placed *Canis lupus baileyi* basal to all other wolf clades, while the five

1238 other North American haplotypes sorted into three different clades that each included Eurasian

1239 *C. lupus*. There was no obvious geographic pattern in the distribution of these haplotypes, but

1240 the scale of sampling was too coarse for this purpose.

1241   The single haplotype shared by the six individuals of *C. l. baileyi* was unique and was

1242 more similar to certain Eurasian wolves than to other North American *C. lupus*. Vilà et al.

1243 (1999, p. 2099) suggested that *C. l. baileyi* may represent an early invasion of North America by

1244 Eurasian wolves, before the arrival of *C. lupus* with other haplotypes. The phylogenetic analysis

1245 also included the mtDNA control region sequence of a single Texas *C. rufus*. The *C. rufus*

1246 sequence was not found in coyotes, although it was within the coyote clade of the neighbor-

1247 joining tree.

1248   Wilson et al. (2000) examined the origin and taxonomy of the wolves of eastern Canada,

1249 and whether they are the result of hybridization between gray wolves and coyotes as has been

1250 suggested for the red wolf (Roy et al. 1996). Wilson et al. (2000, Figures 5A and 5B, reproduced

1251 here as Figure 9 and Figure 10, respectively) compared the mtDNA control region sequences of

1252 historical wolves (1960-1965) from the vicinity of Algonquin Provincial Park (n = 13) and

1253   southern Ontario (n = 8), Texas coyotes (n = 27), captive *C. rufus* (n = 12), and *C. lupus* from

1254   Manitoba, northern Ontario, northern Quebec and the Northwest Territories (n = 9).

1255   The authors found no gray wolf sequences in historical Algonquin wolves or in captive

1256   *C. rufus*. Moreover, captive red wolves and historical Algonquin wolves exhibited unique

1257   haplotypes not found in either *C. lupus* or coyotes. Phylogenetic analyses revealed a similarity

1258   between the unique haplotypes C1 of Algonquin wolves, C3 found in a single modern Manitoba

1259   wolf and C2 of captive red wolves (Wilson et al. 2000, Figure 5). This group was strongly

1260   separated from the wolves of western and northern Ontario as well as the Northwest Territories.

1261   Other eastern Canadian wolves had haplotypes grouped by the analyses with coyote haplotypes,

1262   although most were not found in western coyotes (Lehman et al. 1991, Wilson et al. 2000).

1263   Wilson et al. (2000) attributed the existence of the unique "coyote-like" haplotypes in

1264   Algonquin wolves and *C. rufus*, as well as the ability of these canids to hybridize with coyotes,

1265   to their having shared a more recent common ancestor with coyotes in the New World rather

1266   than with the more distantly related, Old World-evolved *C. lupus*. Based on this relationship,

1267   they concluded that eastern Canadian wolves should not be considered a subspecies (*C. l. lycaon*)

1268   of gray wolf, but as a full species, *C. lycaon*, closely related to, if not conspecific with *C. rufus*.

1269   If conspecific with *C. rufus*, Wilson et al. (2000) suggested that the name that might best apply

1270   would be *Canis lycaon*, based on "historical taxonomic classifications." Wilson et al. (2000)

1271   maintained that *C. lycaon* ranged into Minnesota, northwestern Ontario, and Manitoba, and

1272   suggested that *C. lycaon* and *C. lupus* may be interbreeding in these western reaches.

1273   Eight *C. lupus* individuals from five different North American localities were also

1274   characterized in this study. Although three haplotypes (i.e., C22, C23 and C24) were found, the

1275   scale of sampling was too coarse to detect any obvious geographic pattern in their distribution.

1276   For example, Ontario and Manitoba wolves should represent *C. l. nubilus* but haplotype C23 was

1277   found there as well as in the Northwest Territories where wolves represent *C. l. occidentalis*

1278   (Nowak 1995, Table 4 in this paper). As discussed in the preceding section, Wilson et al. (2000)

1279   also characterized microsatellite variation in wolves from across Ontario, Quebec and western

1280   Canada.

1281   To test the hypothesis that eastern wolves evolved in North America, Wilson et al. (2003)

1282   obtained mtDNA control region sequences from the hides of two historical wolves, one from

1283   New York state (collected in the 1890s) and the other from Penobscot County, Maine (in the

1284   1880s). Both animals were presumed unaffected by coyote hybridization as they had been

1285   collected prior to the invasion of the eastern U.S. by western coyotes. The authors suggested that

1286   the exhibition of "coyote-like" haplotypes by these animals would indicate a most recent

1287   common ancestor between eastern wolves and coyotes rather than gray wolves.

1288   In both neighbor-joining and maximum parsimony analyses (Wilson et al. 2003, Figure

1289   1) neither specimen clustered with *C. lupus* samples from northern Ontario, northern Quebec,

1290   Manitoba, the Northwest Territories, or Europe. The haplotype of the Maine individual was

1291   identical to the unique *C. lycaon* haplotype C1 previously identified from Algonquin Provincial

1292   Park wolves. The New York sample clustered with modern western coyotes, although this

1293   haplotype (C13) was not identical to any reported from coyotes and was referred to by the

1294   authors as "coyote-like." The authors interpreted these findings as supporting both the presence

1295   of *C. lycaon* in Maine and New York in the 1880s and their earlier contention (Wilson et al.

1296   2000) that *C. lycaon* and *C. rufus* represent an eastern wolf with an evolutionary history separate

1297   from that of *C. lupus*.

1298   Adams et al. (2003) compared the mtDNA control region sequences of the four matrilines

1299   of the captive red wolf breeding program (n = 8), red wolf-coyote hybrids (n = 40) collected

1300   from the Alligator River National Wildlife Refuge in northeastern North Carolina (Adams et al.

1301   2003, Figure 1), and coyotes collected from locations in California, Kentucky, Nebraska, North

1302   Carolina, Texas and Virginia (n = 66).

1303   The authors found that the four matrilines of the 14 red wolf founders of the captive

1304   breeding program had identical control region sequences, haplotype Cru (same as C2, Wilson et

1305   al. 2000), and when compared to haplotypes observed in coyotes proximal to the red wolf

1306   experimental population (i.e., 14 haplotypes found in North Carolina and Virginia coyotes, n =

1307   50) and the published literature (i.e., 12 coyote haplotypes), Cru was unique. The average

1308   sequence divergence between haplotype Cru and these 26 coyote haplotypes was 3.24% (range =

1309   1.2-10%). The average sequence divergence among these coyote haplotypes was 2.79% (range =

1310   0.3-11%).

1311   In a study designed primarily to explore the pack composition of wolves in Algonquin

1312   Provincial Park, Grewal et al. (2004) reported on the variation of mtDNA control region

1313   sequence haplotypes (n = 261), Y-chromosome microsatellite haplotypes (n = 248) and

1314   autosomal microsatellite genotypes (n = 261) among wolves at various locations in Ontario and

1315   Quebec. The data from the autosomal and Y-chromosome microsatellite portions of this study

1316   are discussed in the preceding and following sections.

1317   Mitochondrial DNA haplotypes were identified as being of *C. lupus* (C22), *C. lycaon*

1318   (C1, C9), or coyote (C13, C14, C16, C17, C19; but see the summary of the study of mtDNA

1319   variation of Wheeldon and White (2009) for an alternative interpretation of C13). The species

1320   source of the rare haplotypes C3 and C36 were not identified. *Canis lupus* mtDNA haplotypes

1321   were uncommon (four of 102 individuals) in wolves at Algonquin Provincial Park, but were 10

1322   times more common to the north (La Verendrye Reserve) and west (northeastern Ontario) of

1323   Algonquin Park (Table 4 of this paper). *Canis lycaon* mtDNA haplotypes were observed at

1324   higher frequency and coyote haplotypes at lower frequency to the south (Frontenac Axis) of

1325   Algonquin Provincial Park (Grewal et al. 2004, Figure 2, Table 1). Grewal et al. (2004) suggest

1326   that coyote haplotypes probably introgressed into Algonquin Park wolves during the 1960s when

1327   the population was reduced and coyote/wolf hybrids ("Tweed wolves" of Kolenosky and

1328   Standfield 1975) expanded into the park.

1329   Leonard et al. (2005) obtained mtDNA control region sequences from 34 museum

1330   specimens of *C. lupus* collected from the conterminous U.S. and Labrador prior to the extirpation

1331   of wolves (i.e., 1917 or earlier) to determine the proportion of the variation that has been retained

1332   by extant populations of wolves in Alaska, Canada, and northeast Minnesota. The sample of

1333   historical wolves included individuals identified morphologically and by geographic provenance

1334   as *C. l. baileyi* (n = 8), *C. l. labradorius* (n = 4), *C. l. nubilus* (n = 16), and *C. l. youngi* (n = 6).

1335   The 13 mtDNA haplotypes observed in these historic wolves were sorted into northern and

1336   southern clades by phylogenetic analysis. The northern clade, representing *C. l.*

1337   *nubilus/youngi/labradorius* (combined under *C. l. nubilus*, Nowak 1995), included nine

1338   haplotypes, three of which are common in modern wolves (i.e., lu28, lu32 and lu38). The

1339   southern clade, representing *C. l. baileyi*, included four haplotypes, only one of which remains in

1340   modern Mexican wolves (i.e., lu33). An historical *C. l. baileyi* sample from Chihuahua, Mexico,

1341   (USNM98313/JAL474) exhibited an mtDNA haplotype (lu60) that is otherwise known only

1342   from coyotes. Overall, 58% of the historical wolf samples exhibited the same four haplotypes

1343   observed in the modern wolf sample. Eight haplotypes found in historical wolves were not

1344 observed in modern wolves. Individuals with southern clade haplotypes were found as far north

1345 as Utah, Colorado, and Nebraska, whereas, northern haplotypes were found as far south as

1346 Arizona. The authors explained this observation as genetic evidence of population interaction:

1347 "In highly mobile species, large zones of intergradation may characterize subspecies boundaries .

1348 . ."

1349   Musiani et al. (2007) used variation in mtDNA control region sequence, and Y-

1350 chromosome and autosomal microsatellites to examine the effects of habitat specialization on

1351 population structure in wolves from northern Canada. The autosomal and Y-chromosome

1352 microsatellite results are discussed in respective sections.

1353   Mitochondrial DNA control region haplotype frequency and diversity were analyzed in

1354 gray wolves from tundra/taiga (n = 337) habitat in Northwest Territories, and boreal forest

1355 habitat (n = 67) in Northwest Territories and northern Alberta. Nine different haplotypes were

1356 characterized, two predominated in tundra/taiga wolves (89% of sample). While eight

1357 haplotypes were found in boreal forest wolves, the two most common comprised only 42% of the

1358 sample (Musiani et al. 2007, Figure 5). Haplotype frequencies differed between habitat types.

1359 For example, haplotype lu32 was observed in both samples but was far more common among

1360 tundra/taiga wolves (71% of sample) than in boreal forest wolves (22% of sample). Similarly,

1361 mitochondrial gene diversity was three times higher in wolves from boreal forest habitat than in

1362 tundra/taiga wolves (0.051 and 0.153 respectively). Genetic differentiation between wolves of

1363 different sexes from boreal forest and tundra/taiga habitats was three times higher for females

1364 (F_{ST} = 0.353) than males (F_{ST} = 0.138). Consistent with the autosomal microsatellite analysis,

1365 the mtDNA control region sequence data also supported the characterization of boreal forest and

1366 tundra/taiga wolves as separate populations (but see results obtained from Y-chromosome

1367 microsatellite variation). The authors concluded that sex bias, likely due to natal habitat-biased

1368 dispersal, had contributed to the population differentiation reflected by mtDNA variation (F_{ST} =

1369 0.28).

1370   Leonard and Wayne (2008) investigated the controversy surrounding wolf-coyote

1371 hybridization and its impact on the recovery success of western Great Lakes wolves. They

1372 compared mtDNA control region sequences from historical wolves (n = 12, collected 1905-

1373 1916) and 18 modern wolves (n = 18) from portions of Michigan, Wisconsin, and Minnesota.

1374 All twelve historical specimens exhibited what the authors termed "Great Lakes" haplotypes

1375 (i.e., GL1, GL2, GL3, GL5, and GL8) that were basal to modern coyote haplotypes in a

1376 parsimony consensus cladogram (Leonard and Wayne 2008, Figure 1). No historical sample

1377 exhibited a haplotype from the clade that included western gray wolf haplotypes. By

1378 comparison, the composition of the haplotypes in the modern samples was: Great Lakes: 50%;

1379 *C. lupus*: 6%; coyote-like: 44%. In the modern samples, coyote-like haplotypes occurred in

1380 Minnesota and Michigan, but the Michigan sample was small (n = 2) and no modern Wisconsin

1381 wolves were sampled. Both *C. lupus* and Great Lakes haplotypes were found in modern

1382 Minnesota wolves. The authors concluded that historical Great Lakes wolves were characterized

1383 by phylogenetically distinct haplotypes representing an endemic "American wolf" and that these

1384 had been replaced by a modern population of mixed *C. lupus* and coyote ancestry.

1385   Hailer and Leonard (2008) explored the relationships of three canid species, historically

1386 sympatric in central Texas, for evidence of hybridization with an analysis of mtDNA control

1387 region sequence and Y-chromosome microsatellite haplotypes. The Y-chromosome data are

1388 discussed in a following section. The sample included wolves from the captive breeding

1389 programs for *C. l. baileyi* (n = 16) and *C. rufus* (n = 5) as well as wild *C. latrans* from Texas (n =

1390   53) and Nebraska (n = 75). These were compared to each other and with similarly characterized

1391   reference animals from previously-published studies (Vilà et al. 1999, Adams et al 2003,

1392   Leonard et al. 2005).

1393   All *C. rufus* had coyote-like control region haplotype ru1 (Vila et al. 1999, same as Cru

1394   in Adams et al. 2003) that was distinct from *C. l. baileyi*. Although possibly of coyote origin,

1395   haplotype rul was not observed in any of the coyotes (n = 131) in the study. The lu60 haplotype

1396   found in an historical *C. l. baileyi* skull (Leonard et al. 2005) differed by two base changes from

1397   the Texas coyote haplotype la86 and was interpreted as possible evidence of past coyote

1398   introgression into *C. l. baileyi*. A single Texas coyote exhibited the mtDNA haplotype Cla12

1399   which was phylogenetically most similar to the gray wolf haplotype lu32 (Figure 2, Hailer and

1400   Leonard 2008). Haplotype lu32 has also been observed in historical Mexican wolves (Leonard

1401   et al 2005) and the authors suggested that introgression between Mexican wolves and coyotes

1402   may have been the route by which this marker entered the Texas coyote population.

1403   Wheeldon and White (2009) addressed the attestation (Leonard and Wayne 2008) that

1404   phylogenetically distinct historical Great Lakes wolves have been replaced by a modern

1405   population of mixed *C. lupus* and coyote ancestry. In addition to the microsatellite DNA data

1406   summarized previously, Wheeldon and White (2009) compared the mtDNA control region

1407   sequences of three historical specimens (1899-1908) from Minnesota and Wisconsin. The

1408   Wisconsin specimen displayed the *C. lycaon* C1 haplotype identified by Wilson et al. (2000) in

1409   Algonquin wolves, whereas the two Minnesota specimens exhibited the coyote-like C13

1410   haplotype also found in a historical (circa 1890s) wolf hide from New York (Wilson et al. 2003).

1411   Wilson et al. (2003) and Wheeldon and White (2009) considered C13 to be a *C. lycaon*

1412   haplotype related to coyote haplotypes through introgression either before European settlement

1413   or much earlier in the Pleistocene. Both haplotypes were identical to those observed in other

1414   historical wolves from the Great Lakes region (Koblmüller et al. 2009a, Figure 2a). Wheeldon

1415   and White (2009) interpreted these data as supporting an historical western Great Lakes wolf

1416   population with genetic composition similar to the wolves currently populating the region. The

1417   authors' analyses of autosomal microsatellite DNA data are discussed in the preceding section.

1418   Wheeldon et al. (2010) analyzed species-specific mtDNA sequence haplotypes and Y-

1419   chromosome and autosomal microsatellite variation in 410 wolves and coyotes from the western

1420   Great Lakes states (Minnesota, Michigan, and Wisconsin) and western Ontario. The Y-

1421   chromosome and autosomal microsatellite data are discussed separately.

1422   Both eastern wolf and gray wolf haplotypes were found in the wolf sample, however, no

1423   coyote haplotypes were found. The authors used the species attribution criteria of Wilson et al.

1424   (2000) to describe the observed haplotypes. In the combined sample of western Great Lakes

1425   states wolves from Minnesota, Wisconsin and Michigan (n = 203), *C. lycaon* mtDNA control

1426   region sequence haplotypes C1, C3 and C13 were more prevalent (n = 134, 66%) than *C. lupus*

1427   haplotypes C22, C23 and C97 (34%). In contrast, wolves from northern Ontario (n = 135) more

1428   commonly exhibited *C. lupus* haplotypes (56%) than *C. lycaon* haplotypes (44%). Geospatial

1429   maps of the distribution of *C. lycaon*, *C. lupus* and *C. latrans* mtDNA haplotypes revealed that

1430   they occur together throughout the western Great Lakes region (Wheeldon 2010, Fig. 1).

1431   Furthermore, 44% of male western Great Lakes states wolves (n=43) displayed both *C.*

1432   *lupus* and *C. lycaon* mtDNA and Y-chromosome markers. The authors concluded that the

1433   wolves of the western Great Lakes states region are hybrids of gray wolves *C. lupus* and eastern

1434   wolves *C. lycaon*. Unlike *C. lycaon* in southeastern Ontario, which has hybridized extensively

1435   with coyotes (Grewal et al. 2004, Rutledge et al. 2010b), no wolves were found to exhibit coyote

1436   mtDNA haplotypes and only one coyote was found with a wolf mtDNA haplotype (Wheeldon et

1437   al. 2010, Table 1).

1438   Koblmüller et al. (2009a) addressed questions regarding the evolutionary origin of Great

1439   Lakes wolves as well as historical and ongoing hybridization between Great Lakes wolves,

1440   western gray wolves and coyotes. Koblmüller et al. (2009a) compared the mtDNA control

1441   region sequence haplotypes of modern and historical (n = 19, 1892-1916) wolves from the Great

1442   Lakes region as well as reference populations of coyotes and western gray wolves (*C. lupus*).

1443   Although these data were not presented in such a way as to be able to determine the respective

1444   haplotype frequencies observed in wolves from the various modern (n = 6) and historical (n = 7)

1445   Great Lakes region sampling localities, the authors summarized their results with a phylogenetic

1446   analysis (neighbor-joining tree), and identified coyote (containing all coyote individuals) and

1447   wolf clades (containing all western *C. lupus* individuals). The analysis placed most Great Lakes

1448   wolves (n = 142) in the coyote clade, but they were also well-represented in the wolf clade (n =

1449   75). The authors concluded that Great Lakes wolves are gray wolves (*C. lupus*) that have been

1450   influenced by repeated hybridization with coyote in both ancient and recent times. They also

1451   concurred with the conclusions of Leonard and Wayne (2008) that historical Great Lakes wolves

1452   were characterized by phylogenetically distinct haplotypes representing an endemic "American

1453   wolf" and that these have been replaced by a modern population of mixed *C. lupus* and coyote

1454   ancestry. The autosomal and Y-chromosome microsatellite DNA data obtained in this study are

1455   summarized elsewhere.

1456   Muñoz-Fuentes et al. (2009, Figure 3, which is reproduced here as Figure 11) observed a

1457   pronounced differentiation in mtDNA control region sequence variation between coastal and

1458   inland wolves in British Columbia and other localities in western Canada and Alaska. They

1459 attributed this distinction to local discontinuities in vegetation cover and prey composition and

1460 preferences of dispersing wolves for habitats similar to where they were reared.

1461   Although genetic differentiation between all localities was significant, coastal and inland

1462 populations were most different and haplotype frequency and composition among coastal wolves

1463 were markedly different (Table 4 of this paper). Differentiation between historical samples of *C.*

1464 *l. nubilus* (n = 19, Leonard et al. 2005) and modern *C. l. occidentalis* (n = 118, Muñoz-Fuentes et

1465 al. 2009, Table 2) was high (Φ_{ST} = 0.124, where Φ_{ST} is a measure of DNA haplotype divergence

1466 [Excoffier et al. 1992]), but differentiation between coastal British Columbia wolves (n = 75)

1467 within the range of *C. l. nubilus* in Nowak (1995) and historical *C. l. nubilus* was even greater

1468 (Φ_{ST} = 0.550). Muñoz-Fuentes et al. (2009) observed that this differentiation supported the

1469 recognition of coastal British Columbia wolves as a discrete management unit (Moritz 1994).

1470   Assessments of mtDNA control region sequence variation by Muñoz-Fuentes et al.

1471 (2010) detected evidence of wolf-dog hybridization in wolves that recolonized Vancouver Island

1472 after extirpation in the 1950s. The single mtDNA haplotype lu68 found in all 13 available pre-

1473 extirpation specimens (collected 1910-1950) was observed in only 5% of the recovered

1474 Vancouver Island population and 25% of the coastal mainland population (Muñoz-Fuentes et al.

1475 2009). The recovered population primarily exhibited haplotype lu38 (95%), which was also

1476 common (68%) on the mainland coast of British Columbia (Muñoz-Fuentes et al. 2009a).

1477 Although the mtDNA data indicated evidence of dog introgression, the microsatellite data that

1478 were also collected in this study did not (discussed in the preceding section). These results were

1479 found to be consistent with a rare hybridization event that occurred early in the re-establishment

1480 of wolves on the island.

1481   Rutledge et al. (2010a) tested the hypothesis that hundreds of years before the eastward

1482   expansion of coyotes, the wolf of the temperate forests of eastern Canada was a gray wolf. Four

1483   historical (circa 1500 to 1600) *Canis* skull fragments (i.e., three teeth and a lower mandible with

1484   two in situ teeth) were obtained from a pre-European contact Iroquois village archaeological site

1485   in southern Ontario, Canada. None of the remains exhibited gray wolf mtDNA, but tooth

1486   samples L1Va3 and L1Va5 had mtDNA sequence haplotypes previously found in ancient old

1487   world dogs (Vila et al 1997, Leonard et al. (2002), and the remaining tooth L1Va4 and mandible

1488   L1Va6 exhibited mtDNA haplotypes similar to coyotes from Saskatchewan (Rutledge et al.

1489   2010a, Figure 5). Tooth sample L1Va4 had been found in context with the mandible L1Va6, and

1490   morphometric analysis identified this bone specimen as being outside the size range of coyotes

1491   and likely of eastern wolf origin. The authors concluded that the combined genetic and

1492   morphometric data suggest that the archaeological remains are of eastern wolf, *C. lycaon*, origin

1493   and that the historical wolf of eastern Canada was not the gray wolf *C. lupus*. However, they

1494   added that the data were also consistent with a *C. lycaon–C. lupus* hybrid origin (Wheeldon and

1495   White 2009).

1496   Rutledge et al. (2010b) tested the hypothesis that eastern wolves have mediated gene flow

1497   between gray wolves and coyotes in the region of Algonquin Provincial Park, Ontario.

1498   Mitochondrial DNA control region sequence haplotypes were developed for wolves from

1499   northeastern Ontario (n=51), Algonquin Provincial Park (n=127) and Frontenac Axis (n=38).

1500   The autosomal and Y-chromosome microsatellite data also obtained in this study are discussed in

1501   respective sections.

1502   The frequency and composition of the mtDNA haplotypes observed were consistent with

1503   the observations of Wilson et al. (2000) and Grewal et al. (2004) for wolves in Algonquin

1504   Provincial Park (Rutledge et al. 2010b, Table 3). Wolf clade mtDNA haplotypes were observed

1505   at high frequency in northeastern Ontario (53%), low frequency in Algonquin Provincial Park

1506   (7%) and were absent in the southern-most, Frontenac Axis sample. Coyote clade mtDNA

1507   haplotypes attributed to *Canis lycaon* were common in both northeastern Ontario (14%) and

1508   Algonquin Provincial Park (16%), and occurred at high frequency in Frontenac Axis wolves

1509   (61%). Coyote haplotypes were observed at moderate frequency in northeastern Ontario (33%)

1510   and Frontenac Axis (39%) but occurred at highest frequency in Algonquin Provincial Park

1511   wolves (77%; Table 4 of this paper). Female breeders at Algonquin Provincial Park had a higher

1512   frequency of *C. lycaon* haplotypes (36%) than the total sample. These results are in general

1513   agreement with the separation of *C. l. nubilus* and *C. l. lycaon* range along a transect from the

1514   juncture of Lake Superior and Lake Huron eastward to the Quebec border (Nowak 1995).

1515   Fain et al. (2010) examined the taxonomic relationships of wolves in the western Great

1516   Lakes states and tested the influence of coyote hybridization on wolf recovery in the region.

1517   Mitochondrial DNA control region sequence haplotypes were developed for wolves sampled

1518   from Minnesota (n = 42), Wisconsin (n = 65), Upper Peninsula Michigan (n = 17), and western

1519   wolves from Alaska (n = 40), British Columbia (n = 25), and Alberta (n = 25). The study also

1520   included Wisconsin coyotes (n = 132). The autosomal and Y-chromosome microsatellite data

1521   also obtained in this study are discussed in the preceding and following sections, respectively.

1522   The authors found the data from all three marker types to support the recognition of

1523   *Canis lycaon* as a unique species of North American wolf in the western Great Lakes states. The

1524   frequency and composition of the mtDNA haplotypes observed were consistent with the

1525   observations of Grewal et al. (2004) for wolves in northwest Ontario as fully two-thirds of the

1526   combined western Great Lakes states sample exhibited mtDNA haplotypes of *C. lycaon* (see

1527   Fain et al. 2010, Figure 3 for species attribution of haplotypes). Also similar to northwest

1528   Ontario, there was substantial contribution from *C. lupus* as haplotypes lu28 and lu32 were

1529   observed in a third of the population (see Table 4 of this paper).

1530   The geographic scale of the *C. lycaon-C. lupus* hybrid zone was indicated by the

1531   observation of *C. lycaon* mtDNA haplotypes as far west as northwestern Minnesota and *C. lupus*

1532   mtDNA haplotypes in eastern Upper Peninsula, Michigan (Fain et al. 2010, Fig. 2). The *C.*

1533   *lupus*- and *C. lycaon*-derived mtDNA haplotypes observed in western Great Lakes wolves were

1534   exclusive of sympatric *C. latrans* and inconsistent with ongoing hybridization with coyotes.

1535   Stronen et al. (2010) combined morphological characters (body mass and skull features)

1536   and species-specific mtDNA control region sequence haplotypes to assess wolf-coyote

1537   hybridization in wolves from Riding Mountain National Park (n = 19) and Duck Mountain

1538   Provincial Forest (n = 8) in Manitoba. Additional wolf samples from northern Manitoba (n = 13)

1539   and Prince Albert National Park, Saskatchewan (n = 24) were characterized for mtDNA

1540   haplotype only.

1541   The eastern wolf mtDNA haplotype C3 was observed in 50% of the Duck Mountain

1542   Provincial Forest sample but was not observed elsewhere in Manitoba or Saskatchewan (see

1543   Stronen et al. 2010, Figure 3, for species attribution of haplotypes). The *C. lupus* haplotypes

1544   C22 and C23 predominated in Manitoba (25% and 63% respectively) whereas only *C. lupus*

1545   haplotypes C23 and 16 were observed in wolves from Prince Albert National Park,

1546   Saskatchewan (17% and 83% respectively). This study provided further definition to eastern

1547   wolf range; the authors concluded that the western range of eastern wolves did not extend to

1548   Saskatchewan (Table 4 of this paper).

1549   In a previous study of Alaskan wolves, Weckworth et al. (2005) found that coastal and

1550   continental groups were distinguished by significant differences in allele frequencies at

1551   autosomal microsatellite loci, a criterion used to identify management units for conservation

1552   (Moritz 1994). Additional criteria are the exhibition of significant differences in mtDNA

1553   haplotype frequency for management units or reciprocal monophyly in the inheritance of

1554   mtDNA variants by the members of an evolutionarily significant unit. To test for compliance

1555   under these criteria, Weckworth et al. (2010) evaluated the mtDNA of the same individuals

1556   characterized in their earlier study.

1557   Haplotype variation was assessed in coastal southeast Alaska wolves (n = 129), coastal

1558   Gulf of Alaska wolves (n = 73) and wolves from interior localities in Alaska (n = 64), Yukon (n

1559   = 12), and British Columbia (n = 24). Although the mtDNA haplotypes identified in this study

1560   comprised a portion of the cytochrome *b* gene, tRNAs for proline and threonine as well as the

1561   control region, only the control region portion was used in this review. The authors observed a

1562   pronounced differentiation in mtDNA control region sequence variation between coastal wolves

1563   in the southeast archipelago of Alaska and coastal wolves from the Kenai Peninsula and Copper

1564   River delta in the Gulf of Alaska. Similar differences were found in comparisons to populations

1565   in interior Alaska and British Columbia. Although genetic differentiation among all localities

1566   was significant, differentiation between coastal and inland populations was greatest and

1567   haplotype frequency and composition among coastal wolves was unique. As evidence,

1568   haplotype H (Weckworth et al. 2010) also known as lu68 (Muñoz-Fuentes et al. 2009, 2010) is

1569   unique to southeast Alaska and coastal British Columbia (Table 4 of this paper). The authors

1570   attributed this distinction to independent evolutionary histories for coastal and continental

1571   wolves in North America.

1572

1573   *Summary of studies of mtDNA.* The initial interpretation of mtDNA variation in the Great Lakes

1574   region as resulting from coyote-wolf introgression (Lehman et al. 1991) was reinterpreted by

1575   Wilson et al. (2000) and in later studies (Fain et al. 2010, Wheeldon et al. 2010) as the result of

1576   gray wolf-eastern wolf hybridization. Regardless of disagreements over the identity of the taxa

1577   involved, there is general agreement that there was a unique historical wolf population in the

1578   Great Lakes region that has subsequently been affected by hybridization (Wilson et al. 2000,

1579   2003; Leonard and Wayne 2008; Koblmüller et al. 2009a; Fain et al. 2010; Wheeldon et al.

1580   2010).

1581   Early reports indicated red wolves had either gray wolf or coyote mtDNA and

1582   cytochrome *b* gene haplotypes (Wayne and Jenks 1991, Roy et al. 1996). However, surviving

1583   red wolves all have a mtDNA control region haplotype unique to red wolves (Wilson et al. 2000,

1584   Adams et al. 2003, Hailer and Leonard 2008).

1585   The initial observation (Wilson et al. 2000) of the phylogenetic clustering of red wolves

1586   and eastern wolves has not been found in subsequent studies (Wilson et al. 2003, Leonard and

1587   Wayne 2008, Fain et al. 2010).

1588   There is agreement on the phylogenetic uniqueness of the mtDNA control region

1589   haplotypes of historical and contemporary Mexican wolves (Vilà et al. 1999, Leonard et al.

1590   2008), but a single exception is discussed in the Analysis and Discussion.

1591

1592   *Y-chromosome DNA.* In a study designed primarily to explore the pack composition of wolves in

1593   Algonquin Provincial Park, Grewal et al. (2004) reported on Y-chromosome microsatellite

1594   haplotype variation among wolves (n=248) at various locations in Ontario and Quebec.

1595 Haplotypes were developed for the Y-linked microsatellite loci MS41A, MS41B, MS34A,

1596 MS34B characterized by Olivier et al. (1999). Sixteen different haplotypes were identified

1597 (Table 5 of this paper), but species assignments were not made for any of the observed

1598 haplotypes. Four to eight haplotypes were observed in each of the six locality samples. No

1599 haplotype was common to all localities, but the haplotypes AA and CE were each found at five

1600 localities. Seven haplotypes were specific to single localities.

1601 Consistent with the separation of *C. l. nubilus* and *C. l. lycaon* range (Nowak 1995),

1602 differentiation between wolf populations from northern (i.e., northeastern Ontario, Abitibi-

1603 Temiscamingue Region, La Verendrye Reserve) and southern (i.e., Algonquin Provincial Park,

1604 Frontenac Axis, Magnetawan Region) localities was high (Φ_{ST} ranged 0.111 to 0.325), while

1605 differentiation of wolves from within the respective regions was low (Φ_{ST} ranged -0.057 to

1606 0.073).

1607 Bannasch et al. (2005) used paternally inherited Y-chromosome haplotypes from the Y-

1608 linked microsatellite loci MS34A, MS34B and MS41B (Olivier et al. 1999), and 650-79.2, 650-

1609 79.3 and 990-35 described in their own study, to evaluate the genetic structure of purebred dogs.

1610 A total of 824 unrelated male domestic dogs from 50 different breeds were analyzed. Sixty-

1611 seven different haplotypes were observed and many provided genetic distinction between breeds.

1612 Twenty-six breeds exhibited breed-specific haplotypes indicating lengthy independent histories.

1613 Y-chromosome haplotype sharing between a number of breeds reflected a shared origin, and

1614 many of these relationships were confirmed by known breed histories. Neither wolves nor

1615 coyotes were included in this study.

1616 Sundqvist et al. (2006) characterized Y-chromosome haplotype variation in a study of the

1617 origin of domestic dog breeds. Four Y-linked microsatellite loci (Olivier et al. 1999) were

1618 analyzed in wild gray wolves from Alaska (n = 12) and Northwest Territories (n = 13) . Eleven

1619 different haplotypes were observed, only one of which (H32) was found in both samples (Table 5

1620 in this paper).

1621   Musiani et al. (2007) analyzed Y-chromosome haplotype variation in male gray wolves

1622 from boreal forest (n = 32) and tundra/taiga (n = 170) habitat in Alberta and Northwest

1623 Territories, Canada (n = 202). Four Y-linked microsatellite loci (Olivier et al. 1999) were

1624 analyzed, nineteen different haplotypes were observed and nine occurred in > 5% of the sample

1625 (Table 5 in this paper). Although haplotype frequencies differed between habitat types, genetic

1626 differentiation (F_{ST} = 0.03) was not significant between male boreal forest and tundra/taiga

1627 wolves, contrary to the significant differentiation in mtDNA (F_{ST} = 0.28) observed between

1628 habitat types. In addition, Y-chromosome gene diversity was similar in wolves from boreal

1629 forest and tundra/taiga habitat (i.e., 0.897 and 0.891 respectively). In contrast to results obtained

1630 in this study from mtDNA control region sequence and autosomal microsatellite variation

1631 (discussed in previous sections), the Y-chromosome haplotype data did not support the

1632 characterization of boreal forest and tundra/taiga wolves as separate populations.

1633   Hailer and Leonard (2008) assessed cross-species introgression in red wolf, Mexican

1634 wolf, and Texas coyotes using Y-chromosome microsatellite haplotypes from four Y-linked

1635 microsatellite loci (Olivier et al. (1999) as species markers. The sample included male wolves

1636 from the captive breeding programs for *C. l. baileyi* (n = 16) and *C. rufus* (n = 5) as well as male

1637 *C. latrans* from Texas (n = 34) and Nebraska (n = 36). These were compared to each other and

1638 with similarly characterized reference gray wolves (n = 226) from previously-published studies

1639 (Sundqvist et al. 2001, 2006; Musiani et al. 2007).

1640   Two different Y-haplotypes were identified among *C. rufus*, the coyote-like type H15

1641 that was also observed in 6% of the Texas coyote sample, and H1 that the authors recognized as

1642 wolf-like and most similar to haplotypes H28 and H29 found in captive Mexican wolves (Hailer

1643 and Leonard 2008, Figure 3). They suggested that hybridization with Mexican wolves may have

1644 been the route by which this marker entered Texas red wolves. Haplotype H28 was also found in

1645 wolves from Alaska (equals haplotype G, Table 5), but both H1 and H29 were unique among

1646 wolves (Table 5 in this paper) and coyotes.

1647   A total of 59 unique Y-chromosome haplotypes were identified in the Texas and

1648 Nebraska coyote samples, but only three haplotypes were common to both samples. A single

1649 Texas coyote exhibited haplotype H2, which was phylogenetically most similar to gray wolf

1650 haplotype H44 (Hailer and Leonard 2008, Figure 3). Although this haplotype was also found in

1651 a Northwest Territories wolf (Musiani et al. 2007), it may also have been present in historical

1652 wolves through their southern range. The authors suggest that introgression between Mexican

1653 wolves and coyotes may have been the route by which this marker entered the Texas coyote

1654 population.

1655   Koblmüller et al. (2009a) also studied Y-chromosome haplotype variation in wolves of

1656 the Great Lakes region. Y-chromosome haplotypes from the four Y-linked microsatellite loci

1657 characterized by Olivier et al. (1999) and 650-79.3 and 990-35 characterized by Bannasch et al.

1658 (2005) were analyzed in male gray wolves (n = 30) and Great Lakes wolves (n_{Modern} = 111,

1659 $n_{Historic}$ = 4). The autosomal microsatellite and mtDNA sequence data also obtained in this study

1660 are discussed in preceding sections. It was not possible to determine from the presented

1661 information how the observed haplotype frequencies were distributed across the different

1662   collection localities in the study (i.e., Minnesota, Wisconsin and Michigan, U.S., and Northwest

1663   Territories, Alberta, Ontario, and Quebec, Canada).

1664   Seventeen different haplotypes were observed in the western gray wolf sample, 41 in the

1665   Great Lakes wolf sample, with nine haplotypes common to both samples (Koblmüller et al.

1666   2009a, Table 2). None of these haplotypes were named, nor did the authors provide size

1667   information about the alleles from which the haplotypes were compiled. As a result, it was not

1668   possible to equate the Y-chromosome haplotypes identified by Koblmüller et al. (2009a) with

1669   those identified in other similar studies (Table 5 in this paper). A median-joining network of Y-

1670   chromosome haplotypes identified a major divide between coyote and wolf clades. Nearly all

1671   coyotes were found in the coyote clade and all western wolves occurred in the wolf clade, but

1672   haplotypes of Great Lakes wolves were distributed among both clades. The four historical Great

1673   Lakes wolves for which Y-chromosome haplotypes were determined were all from the Upper

1674   Peninsula of Michigan (J. Leonard, Uppsala University, personal communication). The analysis

1675   placed all of these specimens in the wolf clade, suggesting that *C. lupus* was interbreeding with

1676   Great Lakes wolves prior to their near-extirpation (also see Wheeldon & White 2009, Rutledge

1677   et al 2010a).

1678   Rutledge et al. (2010b) tested the hypothesis that eastern wolves have mediated gene flow

1679   between gray wolves and coyotes in the region of Algonquin Provincial Park, Ontario.   Y-

1680   chromosome microsatellite haplotypes were developed from four Y-linked microsatellite loci

1681   (Olivieri et al. 1999) for wolves from northeastern Ontario (n=26), Algonquin Provincial Park (n

1682   = 53), and Frontenac Axis (n = 19). The autosomal microsatellite and mtDNA sequence data

1683   also obtained in this study are discussed in preceding sections.

1684   The frequency and composition of the Y-chromosome haplotypes observed were

1685   consistent with the observations of Grewal et al. (2004, Table 2) for wolves in Algonquin

1686   Provincial Park (Rutledge et al. 2010b, Table 4). The authors used the species assignment

1687   method of Shami (2002) to describe the observed haplotypes. *Canis lupus* Y-chromosome

1688   haplotypes occurred at high frequency in northeastern Ontario (96%) and moderate frequency

1689   among Algonquin Provincial Park (17%) and Frontenac Axis wolves (21%). *Canis lycaon* Y-

1690   chromosome haplotypes were common in both Algonquin Provincial Park (75%) and Frontenac

1691   Axis (47%) but only one wolf from northeastern Ontario (4%) exhibited a *C. lycaon* Y-

1692   chromosome haplotype.   Breeding males at Algonquin Provincial Park had the highest

1693   representation (88.9%) of *Canis lycaon* haplotypes. *C. latrans* haplotypes were common in the

1694   Frontenac Axis sample (32%), rare in Algonquin Provincial Park wolves (8%) and were absent

1695   among northeastern Ontario wolves.

1696   Similar to the results from mtDNA comparisons, the Y-chromosome results also agreed

1697   with the separation of *C. l. nubilus* and *C. l. lycaon* range along a transect from the juncture of

1698   Lake Superior and Lake Huron eastward to the Quebec border (Nowak 1995). Y-chromosome

1699   haplotypes of *C. lupus* origin were five-fold more frequent in northeastern Ontario than to the

1700   south where *C. lycaon* haplotypes predominated.

1701   Fain et al. (2010) analyzed Y-chromosome and mtDNA haplotypes in addition to

1702   autosomal microsatellite variation to evaluate various genetic aspects of the recovered wolf

1703   population in the western Great Lakes states. Chief among these was their species distinction.

1704   The mtDNA and autosomal microsatellite data are discussed in preceding sections.

1705   The Y-chromosome-linked microsatellite loci characterized by Olivier et al. (1999) and

1706   650-79.2A, 650-79.2B, and 990-35 characterized by Bannasch et al. (2005) identified species-

1707 specific Y-chromosome haplotypes in wolves and coyotes from Minnesota, Wisconsin, and

1708 Michigan (Upper Peninsula). Haplotypes were designated as being of either *C. lupus* or *C.*

1709 *lycaon*/*C. latrans* origin after the allele sizing method of Hailer & Leonard (2008). In the

1710 combined sample of male western Great Lakes states wolves (n = 61), half exhibited unique *C.*

1711 *lycaon* haplotypes and the other half *C. lupus* haplotypes (Fain et al. 2010, Table 1). A median-

1712 joining network identified a major divide between coyote and wolf haplotypes (Fain et al. 2010,

1713 Figure 4, which is reproduced here as Figure 12). The 30 coyote Y haplotypes observed were all

1714 in their own clade, the fourteen *C. lupus* Y haplotypes were placed together in a second clade,

1715 and the five Y haplotypes unique to Great Lakes wolves were primarily distributed in a third

1716 clade intermediate between gray wolves and coyotes. Although Y haplotype FWSClyR was

1717 placed in the coyote clade, the authors interpreted this to be the result of ancient introgression

1718 and that this haplotype was being transmitted as a *C. lycaon* lineage marker.

1719 Forty-six percent of male western Great Lakes states wolves displayed both *C. lupus* and

1720 *C. lycaon* mtDNA and Y-chromosome lineage markers, 41% of males exhibited both *C. lycaon*

1721 mtDNA and Y-chromosome haplotypes, but only 13% exhibited both *C. lupus* mtDNA and Y-

1722 chromosome haplotypes. The authors concluded that the western Great Lakes states wolf

1723 population was composed of gray wolves, *C. lupus*, eastern wolves, *C. lycaon*, and their hybrids.

1724 Moreover, the *C. lupus* and *C. lycaon* derived mtDNA and Y-chromosome haplotypes observed

1725 in western Great Lakes states wolves were exclusive of *C. latrans* and inconsistent with ongoing

1726 hybridization with coyotes.

1727 Wheeldon et al. (2010) evaluated species distinction among the wolves and coyotes of the

1728 western Great Lakes region. They investigated Y-chromosome haplotypes based on four Y-

1729 chromosome-linked microsatellite DNA loci (Olivieri et al. (1999) in 209 male wolves and

1730   coyotes from the western Great Lakes states (Minnesota, Michigan, and Wisconsin) and western

1731   Ontario (Table 5 in this paper). Haplotypes were designated as being of either gray wolf or

1732   coyote/eastern wolf types after the allele sizing method of Hailer & Leonard (2008), 11 were

1733   gray wolf types and 18 were coyote/eastern wolf types. Two of the 29 haplotypes observed were

1734   shared by wolves and coyotes.

1735   Forty-four percent of male Great Lakes states wolves displayed both *C. lupus* and

1736   coyote/eastern wolf lineage markers. Twenty-nine percent of males exhibited both

1737   coyote/eastern wolf mtDNA and Y-chromosome markers, and 27% exhibited both *C. lupus*

1738   mtDNA and Y-chromosome markers. The authors concluded that the wolves of the western

1739   Great Lakes region are hybrids of gray wolves (*C. lupus*) and eastern wolves (*C. lycaon*).

1740   Autosomal microsatellite DNA variation and mtDNA haplotypes from this study were

1741   summarized in previous sections.

1742

1743   *Summary of studies of Y-chromosome DNA.* Studies of wolves in the Great Lakes region have

1744   found haplotypes distributed in either a wolf clade (*C. lupus*), or a clade that includes eastern

1745   wolf and coyote. These studies differ in characterizing eastern wolf haplotypes as alternatively

1746   *Canis lycaon* (Fain et al. 2010, Rutledge et al. 2010a, Wheeldon et al. 2010), Great Lakes wolf

1747   (Koblmüller et al. 2009a), or coyote (Koblmüller et al. 2009a). Studies covering the western

1748   Great Lakes region differ in concluding that wolf-coyote hybridization has been rare (Fain et al.

1749   2010, Wheeldon et al. 2010), or that that it has occurred recently and is currently ongoing

1750   (Koblmüller et al. 2009a).

1751   Studies that presented data on both the mtDNA and Y-chromosome haplotypes of

1752   individual male wolves of the western Great Lakes region agree that the population consists of

1753   gray wolves, eastern wolves, and individuals that are admixed with respect to these two kinds of

1754   wolves (Fain et al. 2010, Wheeldon et al. 2010).

1755   In contrast to the unique, coyote clade mtDNA haplotype of captive red wolves, one of

1756   the two Y-chromosome haplotypes found in red wolves was identical to a haplotype found in

1757   Texas coyotes (Hailer and Leonard 2008), and the other was identical to a haplotype found in

1758   domestic dogs (Bannasch et al. 2005).

1759

1760   **Analysis and Discussion**

1761   Views vary on the number and identity of modern species of *Canis* in North America.

1762   There is general agreement only that coyote is a separate species, and that dogs are derived from

1763   *Canis lupus* (Vilà et al 1997,1999). The predominant view of the taxonomy of those surviving

1764   North American *Canis* known by the general vernacular name of "wolves" follows Goldman

1765   (1944) in recognizing two species: *Canis lupus* (including the subspecies *Canis lupus lycaon*)

1766   and *Canis rufus* (Nowak 1979, 1985; Hall 1981). A variant of this view incorporates the

1767   contention that *Canis rufus* is the result of hybridization between *C. lupus* and coyote (Wayne

1768   and Jenks 1991). Wilson et al. (2000) also recognize two wolf species, but propose that eastern

1769   wolf, *Canis lycaon*, is the same species as *C. rufus*, and that these together are specifically

1770   distinct from *C. lupus*. Baker et al. (2003) recognize three species: *C. lupus*, *C. lycaon*, and *C.*

1771   *rufus*. Wilson and Reeder (2005) include all North American wolves in *C. lupus*, which includes

1772   subspecies *C. l. rufus* and *C. l. lycaon*.

1773   The following analysis and discussion will first address the number of species of wolf in

1774   North America. It will begin with probably the most contentious question of whether the eastern

1775   wolf is within the species limits of *C. lupus*. The taxonomic status of red wolf and eastern wolf

1776   with respect to each other and coyote will then be addressed. Last, the subspecies classification

1777   within *C. lupus* will be evaluated.

1778

1779   **Species limits of *Canis lupus* relative to eastern wolf**

1780   The most contentious issue related to the species limits of *C. lupus* in North America is

1781   the placement of the eastern wolf, which has also been referred to as the Great Lakes wolf

1782   (Leonard and Wayne 2008): Is the eastern wolf within the species limits of *C. lupus* as either a

1783   subspecies, *C. l. lycaon* (Goldman 1937) or a unique ecotype (Koblmüller et al. 2009a), or does

1784   it represent a different species, *C. lycaon*, outside the species limits of *C. lupus*? This section

1785   assesses whether populations referred to as eastern wolves should be considered members of *C.*

1786   *lupus*.

1787   The positions of various authors of taxonomic studies on the geographic range of the

1788   eastern wolf were summarized earlier in the section on taxonomy. All extant wolves that might

1789   be assigned to the eastern wolf occur in the general area from southern Ontario and Quebec, west

1790   to Minnesota and Manitoba. Wolves in this range were nearly exterminated and, by the 1970s,

1791   the only known wolves remaining in the conterminous United States were in northeastern

1792   Minnesota (Figure 1 of this paper, Mech 1974). At about this time, wolves had been eliminated

1793   from southern Ontario and Quebec (Mech 1974) and replaced by a population of coyotes that

1794   had been influenced by hybridization with wolves (Kolenosky and Standfield 1975, Rutledge et

1795   al. 2010b). Wolves have subsequently expanded their range in Minnesota and re-occupied

1796   Wisconsin and the Upper Peninsula of Michigan.

1797   The eastern wolf and gray wolf are known to interbreed within the Great Lakes region.

1798   Where available data permit, the approach here will be to first evaluate divergence between

1799   eastern wolves and gray wolves, then to attempt to evaluate the implications of admixture for

1800   determining potential inter-species barriers.

1801

1802   *Morphometrics.* Trends of increasing size among wolves to the north and west of southern

1803   Ontario and Quebec have been noted in morphometric studies covering the Great Lakes region.

1804   The association of smaller wolves with white-tailed deer in deciduous forests and larger wolves

1805   with larger prey, such as moose and caribou, in boreal forests has been frequently cited

1806   (Kolenosky and Standfield 1975, Skeel and Carbyn 1977, Schmitz and Kolenosky 1985). When

1807   wolf skulls were divided by source habitat into deciduous forest (eastern wolf) and boreal forest

1808   (*C. lupus*), discriminant function analysis distinguished 75% of boreal wolves from eastern

1809   wolves, and boreal wolves were over 25% larger in body mass (Kolenosky and Standfield 1975).

1810   This size difference cannot be entirely attributed to interbreeding of eastern wolves with coyotes

1811   because the "Tweed wolves" of southern Ontario, which have been influenced by recent coyote

1812   introgression, were excluded from these samples. Discriminant function analysis of additional

1813   skull and body measurements have confirmed the smaller size of eastern wolves relative to gray

1814   wolves and distinguish them from coyotes and coyote-wolf hybrids (Schmitz and Kolenosky

1815   1985). Moreover, gray-fawn coat color was most common in both samples, but black, cream,

1816   and white colors found in boreal wolves were rare in eastern wolves.

1817   Skeel and Carbyn's (1977, Figures 2, 3) principal components analysis places eastern

1818   wolves intermediate between *C. l. nubilus* and *C. l. occidentalis* (following Nowak's [1995]

1819   revision) on the first principle component axis, but are closer to *C. l. nubilus*. Their eastern wolf

1820   sample was from extreme southwestern Ontario in an area where influence by *C. l. nubilus* can

1821   be expected.

1822   Nowak's (1979, Figure 7) discriminant function analysis of skull features found the

1823   individuals he attributed to *C. l. lycaon* (eastern wolf) to be generally smaller than northern and

1824   western *C. lupus.* Within this eastern wolf sample, the individuals from the western range that

1825   he recognized for *C. l. lycaon* (Michigan, Minnesota, Wisconsin, and western Ontario) had a

1826   greater range of variation and several individuals were larger than wolves from southern Ontario

1827   and Quebec. Based on this and additional morphometric analyses (Nowak 1995, 2002, 2009),

1828   Nowak recognizes the eastern wolf as a subspecies of *C. lupus* and restricts its range to southern

1829   portions of Ontario and Quebec, while attributing Minnesota wolves to *C. l. nubilus*. His

1830   Minnesota samples, however, were taken after 1960 (Nowak 1995) or 1970 (Nowak 2002,

1831   2009), during a period of likely increased movement into Minnesota of *C. l. nubilus* from the

1832   west and north (Mech and Frenzel 1971, Mech and Paul 2008, Mech 2010, Mech and Nowak, in

1833   review). Nowak's data for Minnesota likely reflect this substantial and recent contribution of *C.*

1834   *l. nubilus* to Minnesota wolves. As will be discussed later, the genetic data (Fain et al. 2010,

1835   Wheeldon et al. 2010) indicate a substantial genetic contribution from the eastern wolf

1836   throughout the western Great Lakes to Minnesota and western Ontario.

1837   The utility of Nowak's (1995) analysis is limited in determining whether the eastern wolf

1838   is distinct from *Canis lupus* by his inclusion of recent wolves of probable *C. l. nubilus* origin in

1839   samples intended to represent the eastern wolf. The sample from Algonquin Provincial Park is

1840   the only eastern wolf sample in these studies that has not been greatly influenced by gray wolves.

1841   When Algonquin wolves are identified as such on discriminant function plots (Nowak 1995,

1842   Figures 10, 11), most individuals appear outside the polygons representing *C. lupus*. Additional

1843   eastern wolves from southern Ontario and Quebec produced a similar result (Nowak 2002,

1844   Figure 8; 2009, Figure 15.2).

1845   The eastern wolf is smaller than other wolves in the analysis, and geographical trends in

1846   size correspond to habitat differences and support the proposal of Kolenosky and Stanfield

1847   (1975) of prey specialization of smaller wolves on white-tailed deer in deciduous forests.

1848   Morphometric data do not provide direct information on monophyly or reproductive

1849   relationships, but in this case the geographic trends in body size are consistent with habitat

1850   distributions and ecological specialization.   All morphometric studies find the eastern wolf to be

1851   an outlier to *C. lupus*, and where there is some overlap in morphometric space, the eastern wolf

1852   extends well beyond the limits of *C. lupus*.

1853

1854   *Microsatellite DNA.* The variably admixed nature of eastern wolf populations in the western

1855   Great Lake states determined from microsatellite DNA analysis has been noted by Fain et al.

1856   (2010) and Wheeldon et al. (2010). The conclusion of Koblmüller et al. (2009a) of little

1857   admixture in wolves of this region may have been influenced by the distant (Northwest

1858   Territories and Alberta) western gray wolf samples used for comparison. STRUCTURE

1859   analysis, as well as divergence measures such as F_{ST}, can be erroneously interpreted as

1860   indicating genetic discontinuities when there are significant gaps in sampling pattern (Pritchard

1861   et al. 2000, Schwartz and McKelvey 2009). Further, these western wolves likely represent *C. l.*

1862   *occidentalis*, rather than *C. l. nubilus*, the subspecies of gray wolf likely to be involved in

1863   admixture with the eastern wolf in the western Great Lakes.

1864   There are indications in the STRUCTURE analysis presented by Koblmüller et al.

1865   (2009a, Figure 3) that there is more genetic variation in the wolf sample from the Great Lakes

1866   region than suggested by the outcome (Figure 3(c)) using $K = 4$. The $L(K)$ values for $K = 4$ and

1867 $K = 5$ are indistinguishable in their Figure 3(a), and the range of variation for Great Lakes

1868 wolves in the factorial correspondence analysis (their Figure 3(d) is much larger than that of

1869 coyotes and western gray wolves. Elevated levels of genetic variation are consistent with an

1870 admixed population.

1871 Wheeldon et al. (2010) included samples from northwest Ontario that clearly portray

1872 admixture, and the higher allelic richness of western Great Lakes wolves (Fain et al. 2010) is

1873 also consistent with their genetically composite ancestry. Data from historical specimens from

1874 Minnesota and Wisconsin (Wheeldon and White 2009, Koblmüller et al. 2009a) also suggest that

1875 admixture of the eastern wolf and western *C. lupus* had taken place prior to their extirpation from

1876 the region.

1877 Both the microsatellite and morphometric data indicate the same trend from Algonquin

1878 Provincial Park in the east with increasing influence by *C. lupus* to the west. In the western

1879 Great Lakes, nearly all wolves have indications of admixture (Fain et al. 2010, Wheeldon et al.

1880 2010), but individual wolves vary greatly in the proportion of eastern wolf influence in their

1881 microsatellite DNA genotypes. In other words, the microsatellite DNA data indicate that the

1882 wolves of the western Great Lakes region do not comprise a homogenous population, which is

1883 consistent with a composite origin and incomplete admixture.

1884 The microsatellite DNA data distinguished eastern wolves from western *C. lupus* in

1885 neighbor-joining trees (Roy et al. 1994, García-Moreno et al. 1996, Wilson et al. 2000),

1886 multidimensional scaling (Roy et al. 1994, García-Moreno et al. 1996), log likelihood (Wilson et

1887 al. 2000), factorial correspondence (Wheeldon et al. 2010), probability of identity (Wilson et al.

1888 2000), and STRUCTURE analyses (Koblmüller et al. 2009a, Wilson et al. 2009, Fain et al. 2010,

1889 Wheeldon et al. 2010). Eastern wolves are also distinguishable in studies that include *C. lupus*

1890 from northern Ontario (Rutledge et al. 2010b).

1891   Despite the expectation that linearity between genetic distance measures and geographic

1892   separation is lost when samples are far apart (Paetkau et al. 1997, Schwartz and McKelvey

1893   2009), genetic distance measures are greater for comparisons between western gray wolves and

1894   eastern wolves than for comparisons between paired samples with substantial eastern wolf

1895   composition (Table 6). There is geographic discontinuity between western gray wolf and eastern

1896   wolf samples in some studies, but greater divergence between eastern wolves and gray wolves is

1897   also found in studies that include grey wolf samples in close proximity to eastern wolves (Roy et

1898   al. 1994, Grewal et al. 2004, Wilson et al. 2009, Rutledge et al. 2010b).

1899   Private alleles are another indication of the relative isolation of populations (Slatkin

1900   1985). These are alleles that are found within a single locality or population, and it is inferred

1901   that they are locally restricted owing to isolation. Information on private alleles in North

1902   American wolf populations are summarized in Table 7. Private alleles are determined only with

1903   respect to other wolf populations; many of these alleles are shared with coyotes. The number of

1904   private alleles is much higher in populations including eastern wolf (southern Quebec and the

1905   western Great Lakes states) than in western gray wolves. Some private alleles are at relatively

1906   high frequencies, which is a further indication of a history of isolation. For example, four such

1907   alleles have frequencies ranging from 0.146 to 0.202 in samples of wolves from the western

1908   Great Lakes states (Fain et al. 2010), consistent with continuing isolation despite a history of

1909   admixture.

1910

1911   *Single nucleotide polymorphisms*. Principal components analysis of the SNP data places the

1912   Great Lakes wolf (eastern wolf) sample closer to gray wolves than to coyotes (vonHoldt 2011,

1913   Figure 3, Supplemental Figure S4). F_{ST} values between the Great Lakes sample and other North

1914   American *Canis* samples range from 0.05 for western gray wolves to 0.11 for Mexican wolf, red

1915   wolf, and coyote. In comparison, F_{ST} values among coyotes from different regions

1916   (midwestern/southern, northeastern, and western) range from 0.02 to 0.05 (vonHoldt et al. 2011,

1917   Supplemental Table S3). Successive STRUCTURE analyses grouped the Great Lakes sample

1918   with wolves, rather than coyotes, beginning at K = 3, although substantial assignment to coyote

1919   ancestry is evident. Assignment of gray wolf ancestry to individual Great Lakes wolves ranged

1920   from about 50% to 100%. vonHoldt et al. (2011) conclude that Great Lakes wolves are

1921   genetically distinct from western gray wolves.

1922   Features of the sample of wolves from the Great Lakes region make it difficult to

1923   evaluate the taxonomic significance of the SNP data. The Great Lakes sample is small (n = 19),

1924   and except for two individuals from Algonquin Provincial Park, their geographic origins within

1925   the region are identified only to the level of province or state. Mitochondrial DNA and Y-

1926   chromosome lineage markers (discussed in the next section) are the primary basis for

1927   recognizing an admixed population including both eastern wolf and gray wolf in the region

1928   (Wilson et al. 2000, Fain et al. 2010, Wheeldon et al. 2010), yet the haplotype composition on

1929   the sample used for SNP analysis is not reported. This sample may include both eastern and gray

1930   wolves, which is consistent with the observation that the proportion of gray wolf ancestry varies

1931   greatly among individuals in the sample. Because eastern wolf is interpreted as having a

1932   common ancestry with coyotes (Wilson et al. 2000), the gray wolf/coyote admixture in the Great

1933   Lakes sample may alternatively represent gray wolf/eastern wolf admixture. Inclusion of gray

1934   wolves in the Great Lakes sample would increase the similarity between the Great Lakes sample

1935   and western gray wolves, just as it has in the morphometric analysis by Nowak (1995). Finally,

1936   the first two axes of the principal components analysis (vanHoldt et al. 2011, Figure 3) account

1937   for relatively little of the variance (10% and 1.7%, respectively); The first principal component

1938   separates dogs from the wild canids, but does separate wolves from coyotes. This separation of

1939   dog from wolves, from which they were derived, indicates that signatures of genealogical history

1940   that might be detectable in this analysis can be labile over the time period since dogs were

1941   domesticated.

1942

1943   *Mitochondrial and Y-chromosome haplotypes.* Studies using these maternally and paternally

1944   inherited lineage markers generally agree that North American *Canis* can be attributed to three

1945   clades: one representing *Canis lupus*, one coyotes, and the third representing members of wolf

1946   populations in the Great Lakes region attributed to eastern wolf (Wilson et al. 2000, Koblmüller

1947   et al. 2009a, Fain et al. 2010, Wheeldon et al. 2010). The current wolf population of the western

1948   Great Lakes includes haplotypes from both *C. lupus* and eastern wolf clades, with eastern wolf

1949   haplotypes predominating. However the unique, "coyote-like" eastern wolf haplotypes of the

1950   Great Lakes region may have originated, there seems to be agreement that they support the

1951   existence of a unique taxon in the region, although the modern population is admixed (Wilson et

1952   al. 2000, Leonard and Wayne 2008, Koblmüller et al. 2009a). There is also disagreement on

1953   whether it should be recognized as a species (Wilson et al. 2000) or as a subspecies (Nowak

1954   1995), and some researchers decline to apply a scientific name and refer to it as an "endemic

1955   American wolf" (Leonard and Wayne 2008) or as a unique ecotype or taxon (Koblmüller et al.

1956   2009a).

1957   Wilson et al. (2000) have proposed that the relatively large lineage divergence in mtDNA

1958   of the eastern wolf from gray wolves to the north and west is sufficient to recognize it as a

1959   separate species. Wilson et al (2000, p. 2159) reported control region mtDNA haplotype

1960 sequence divergence between eastern wolves and western *C. lupus* as 8% (Wilson et al. 2000, p.

1961 2159), compared to the average sequence divergence of 2.9 % among major clades of *C. lupus*

1962 (Vilà et al. 1999). In other studies (summarized in Table 8), sequence divergences between

1963 eastern wolves and western gray wolves are about an order of magnitude larger than within-

1964 species divergences. Using the common estimate of 2% sequence divergence per million years

1965 for mtDNA (Brown et al. 1979), the most divergent eastern wolf haplotypes diverged from

1966 ancestral gray wolf haplotypes about 4 mya, which approaches the estimated age of the genus

1967 *Canis* (Kurtén and Anderson 1980). Within-species sequence divergences this large appear to be

1968 rare in mammals (Avise et al. 1998), and are likely the result of long isolation. Following the

1969 hypothesis of Wilson et al. (2000), a specifically distinct eastern wolf evolved in North America

1970 from a common ancestor with coyotes rather than from a more immediate common ancestor with

1971 *C. lupus*, which evolved in Eurasia (Kurtén and Anderson 1980, Wayne and Vilà 2003).

1972   Both mtDNA and Y-chromosome haplotypes of eastern wolves are members of

1973 monophyletic clades that are highly divergent from those of gray wolves. Because eastern

1974 wolves are phylogenetically more associated with coyotes, inclusion of eastern wolves in the

1975 same species with gray wolves would make the latter paraphyletic with respect to coyotes, which

1976 violates strict phylogenetic species criteria. Alternative explanations for paraphyly of a *C. lupus*

1977 that includes the eastern wolf involve incomplete lineage sorting, and hybridization with coyotes.

1978   Incomplete lineage sorting within a broadly defined *C. lupus* is not a likely explanation

1979 for paraphyly because it is usually associated with relatively recent speciation events, and the

1980 highly divergent haplotypes of eastern wolves and coyotes indicate an ancient divergence. In

1981 addition, putative paraphyly is geographically localized to the Great Lakes region and there is no

1982   evidence of it elsewhere within the large geographic range of overlap between *C. lupus* and

1983   coyotes.

1984   That leaves hybridization as the remaining explanation for the paraphyletic inclusion of

1985   "coyote-like" Y-chromosome and mtDNA haplotypes in *C. lupus*. Koblmüller et al. (2009a)

1986   recognize the eastern wolf as a form of *Canis lupus* that owes its divergent genetic features to

1987   hybridization with coyotes, both ancient and ongoing, rather than to a long period of evolution as

1988   a separate lineage independent of *C. lupus*. A problem with recent coyote introgression as an

1989   explanation for the divergent mtDNA haplotypes of the eastern wolf is that these haplotypes are

1990   not found in coyotes, except those that have recently hybridized with the eastern wolf in southern

1991   Ontario and now occupy the northeastern United States (Kays et al. 2009, Rutledge et al. 2010b).

1992   The coyote-introgression hypothesis requires that the coyotes involved were of Y-chromosome

1993   and mtDNA haplotype clades that have subsequently become extinct. Although there is

1994   evidence, discussed later, of introgression of ancient coyote lineages that are now found only in

1995   the eastern wolf, such instances appear to be rare. The rarity of ancient coyote-eastern wolf

1996   introgression indicates that eastern wolves have been evolving as a separate lineage for a

1997   considerable time.

1998   There is also disagreement among researchers on whether introgression is ongoing

1999   between coyotes and wolves in Minnesota, Wisconsin, and Michigan. Those favoring ongoing

2000   hybridization view western coyotes that have recently moved eastward as the source of the

2001   unique but "coyote-like" mtDNA and Y-chromosome haplotypes in eastern wolves (Lehman et

2002   al. 1991, Leonard and Wayne 2008, Koblmüller et al. 2009a). The contrasting view of little or

2003   no ongoing introgression from coyotes in this area interprets these haplotypes as a consequence

2004   of shared ancestry between coyotes and eastern wolves (Wilson et al. 2000, Wheeldon and White

2005   2009, Fain et al. 2010, Wheeldon et al. 2010). The absence of western coyote Y-chromosome or

2006   mtDNA haplotypes in the current wolf population of Minnesota, Wisconsin, and Michigan is

2007   inconsistent with the hypothesis of ongoing coyote introgression in this area (Fain et al. 2010,

2008   Wheeldon et al. 2010).

2009   The distinctive "coyote-like" mtDNA haplotypes of the eastern wolf could have resulted

2010   from ancient introgression from now-extinct coyote lineages, followed by a selective sweep.

2011   This assumes that the ancestral population of the eastern wolf had haplotypes from the wolf

2012   lineage, and therefore of Old World origin, but that it hybridized with ancient coyotes to such an

2013   extent that the original wolf-lineage mtDNA haplotypes were entirely replaced by coyote

2014   haplotypes. This requires a mating advantage of female coyotes in coyote-wolf mating, or a

2015   strong selective advantage for the mitochondrial genome of coyotes. The general process of

2016   haplotype replacement by a selective sweep is sometimes called cytoplasmic capture or

2017   mitochondrial capture. It has been reported in various plant and animal taxa (Avise 2004, Table

2018   7.6), and can even result in total replacement of the mtDNA of one species by the mtDNA of

2019   another (Nevada et al. 2009).

2020   Distinguishing mitochondrial capture from lineage divergence or incomplete lineage

2021   sorting can be difficult. Although it does occur, it is uncertain whether it occurs often enough to

2022   serve as a general explanation of species-level paraphyly in animal taxonomy. In a review of

2023   paraphyly in bird species, McKay and Zink (2010) found that most cases were the result of

2024   taxonomic errors or incomplete lineage sorting, with few clear instances involving hybridization.

2025   In addition to mtDNA, the eastern wolf differs from coyotes and gray wolf in that it also has

2026   distinguishing morphological, ecological, Y-chromosome, and nuclear autosomal DNA

2027 characteristics. Moreover, there is no indication that such selective sweeps have occurred

2028 elsewhere in North American wolves.

2029 Y-chromosome haplotypes can also be separated into wolf and coyote clades, with the

2030 distinctive haplotypes of eastern wolves basal to the coyote clade (Koblmüller et al 2009a, Fig

2031 2(b); Fain et al. 2010, Figure 12 of this paper). As with mtDNA, inclusion of eastern wolf with

2032 *C. lupus* forms a paraphyletic group with respect to coyotes. An explanation of the high

2033 incidence of coyote-clade, Y-chromosome haplotypes in the eastern wolf through coyote

2034 introgression and displacement of wolf-clade haplotypes requires a selective advantage of coyote

2035 Y-chromosomes or a mating advantage of male coyotes over male gray wolves, which is the

2036 opposite of the usual relationship (Lehman et al. 1991) and seems unlikely.

2037 It is reasonable based on their relative divergence from coyote mtDNA haplotypes to

2038 regard the most strongly divergent eastern wolf mtDNA haplotypes C1 and C3 (Wilson et al.

2039 2000) and Y-chromosome types E, O, Y, and X (Fain et al. 2010) as indications of the initial

2040 divergence from their common ancestor with coyotes, and the less divergent, "coyote-like"

2041 haplotypes mtDNA C13 (Wilson et al. 2003) and Y-chromosome type R (Fain et al. 2010) as

2042 representing subsequent, ancient introgression. The more divergent Y-chromosome haplotypes

2043 of the eastern wolf appear intermediate between *C. lupus* and coyotes in the phylogenetic

2044 assessment of Fain et al. (2010, Figure 12 of this paper). In summary, species-level recognition

2045 of *Canis lycaon*, the eastern wolf, outside the species limits of *C. lupus* is supported by the

2046 phylogenetic distinctiveness of its mtDNA and Y-chromosome haplotypes. Inclusion of the

2047 eastern wolf within *C. lupus* would render the latter paraphyletic with respect to coyotes. The

2048 alternative explanation that these distinctive eastern wolf haplotypes are the result of

2049 introgression of a *Canis lupus* population by coyotes and replacement of wolf-clade haplotypes

2050   by coyote haplotypes is not supported with evidence of these haplotypes in modern coyotes other

2051   than those that have recently hybridized with *C. lycaon* in eastern Canada (Rutledge et al 2010b).

2052

2053   *Conclusions on species limits of Canis lupus relative to the eastern wolf.* Available

2054   information on morphology, ecology, behavior, and genetics are concordant in supporting the

2055   existence of a unique form of wolf native to the Great Lakes region of North America. The

2056   small size of this eastern wolf in comparison to gray wolves is consistent with its association

2057   with white-tailed deer as its preferred prey among ungulate species. Genetic divergence

2058   measures at nuclear microsatellite DNA loci and a relatively high incidence of private alleles

2059   indicate that the eastern wolf has maintained cohesion and has had a long, separate evolutionary

2060   history from gray wolves. Mitochondrial DNA and Y-chromosome haplotypes indicate that

2061   eastern wolves and gray wolves evolved independently as separate lineages for a considerable

2062   time. These concordant lines of evidence and the age of lineage separation indicated by mtDNA

2063   haplotypes support the proposal of Wilson et al. (2000) that the eastern wolf and *Canis lupus*

2064   belong to separate species. The taxonomic interpretation best supported by available mtDNA and

2065   Y-chromosome haplotype data is that the eastern wolf evolved independently from a common

2066   ancestor with coyotes and independently of *C. lupus*.

2067   The eastern wolf, as *Canis lycaon*, has also been recognized as a species-level taxon by

2068   Baker et al. (2003) in a recent revision of their checklist of North American mammals north of

2069   Mexico. The uniqueness of the Great Lakes wolf population, despite admixture, is recognized

2070   even by those who do not favor recognizing it as a separate species (Leonard and Wayne 2008,

2071   Koblmüller et al. 2009a).

91

2072   It is remarkable that strong genetic signatures of separate evolutionary history remain

2073   detectable in the face of modern admixture of eastern wolves and gray wolves in the western

2074   Great Lakes region and eastern wolves and coyotes in the eastern Great Lakes region. It is

2075   notable that wolves from the two lineages have probably been in contact since *C. lupus*, an Old

2076   World lineage, appeared in North America over 500,000 years ago (Kurtén and Anderson 1980),

2077   yet mtDNA haplotypes of historical specimens (Wilson et al. 2003, Leonard and Wayne 2008,

2078   Wheeldon and White 2009, Rutledge 2010a) from the Great Lakes region are eastern wolf or

2079   coyote-like and not *C. lupus*.

2080   The geographic range of the eastern wolf and the extent of its hybrid zone with *C. lupus*

2081   can be mapped using the geographic distribution of the mtDNA and Y-chromosome haplotypes

2082   of the two species (Wilson et al. 2000, 2009; Fain et al. 2010, Wheeldon et al. 2010). The

2083   recognition of the range of eastern wolf extending through Minnesota in the southwest and to

2084   Manitoba in the northwest (Stronen et al. 2010), reestablishes the western portion of the

2085   geographic range of eastern wolf recognized by Goldman (1944) and Hall (1981) for *C. l.*

2086   *lycaon*.

2087   An historical reconstruction leading to the current relationship between the eastern wolf

2088   and *C. lupus* begins with *C. lupus* evolving in Eurasia, while the eastern wolf was evolving in

2089   North America from a common ancestor with coyotes (Wilson et al. 2000, Wheeldon and White

2090   2009). North American *C. lupus* is inferred to have evolved in Eurasia based on fossils (Kurtén

2091   and Anderson 1980) and on the phylogenetic similarity of its haplotypes to certain Eurasian *C.*

2092   *lupus* (Wayne et al. 1995, Vilà et al. 1999, Wayne and Vilà 2003). At this time the eastern wolf

2093   and Eurasian *C. lupus* would have been reciprocally monophyletic, which is indicative of

2094   species-level distinction following strict phylogenetic species concepts and criteria.

2095   When *C. lupus* subsequently invaded North America and came into proximity with the

2096   eastern wolf, the two species may have immediately started hybridizing, at least to some degree.

2097   Ecological isolation has been invoked as an explanation for the persistence of the two species or

2098   kinds of wolves (Standfield 1970). Differences in habitat and prey preference have been found

2099   to significantly affect the genetic structure of wolf populations in North America (Carmichael et

2100   al. 2001, Musiani et al. 2007) and Europe (Pilot et al. 2006). Preference of the eastern wolf for

2101   white-tailed deer in eastern deciduous forest habitats and of *C. lupus* for moose and caribou in

2102   more boreal habitats (Standfield 1970) may have limited encounters between the two species.

2103   However, with human-mediated conversion of boreal forests to deciduous forest and consequent

2104   expansion of white-tailed deer, contacts between the species would have increased. Lowered

2105   population densities of wolves as a result of persecution by people would have decreased the

2106   likelihood of encountering conspecific mates and increased the likelihood of inter-species

2107   matings. This "Allee effect" would have accelerated admixture.

2108   There is little evidence for ongoing or recent hybridization between wolves and coyotes

2109   in the western Great Lakes states, but there are strong indications that admixture of eastern wolf

2110   and coyotes has occurred recently or is ongoing in the eastern Great Lakes region (Wilson et al.

2111   2009, Kays et al. 2009, Rutledge et al. 2010b). The finding of 400-500 year old "wolves" in

2112   archaeological context having coyote and dog mtDNA (Rutledge et al. 2010a) suggests

2113   interbreeding between eastern wolves and coyotes had occurred long before 1919, the earliest

2114   historical record of coyotes in southern Ontario (Nowak 1979, p. 15).

2115   An admixed population of *C. lupus* and the eastern wolf occurs across an area extending

2116   from eastern Ontario to Minnesota and into central Manitoba (Wilson et al. 2000, 2009, Grewal

2117   et al. 2004, Wilson et al. 2009, Fain et al. 2010, Stronen et al. 2010, Wheeldon et al. 2010). The

2118   incidence in Minnesota, Wisconsin and Michigan of 36% of male wolves with the Y-

2119   chromosome marker of one species and the mtDNA marker of the other (Fain et al. 2010)

2120   indicates that inter-species hybridization has occurred. Eastern wolf mtDNA haplotypes are

2121   more common in this area, but the incidence of markers for *Canis lupus* is substantial, especially

2122   in western Minnesota. Y-chromosome (paternal) markers of the two lineages are more evenly

2123   represented.

2124   Admixture between members of long-separate lineages introduces problems in applying a

2125   restrictive standard for reproductive isolation as a criterion for species limits. Templeton (1989)

2126   identified hybridization among species of *Canis* as an example justifying the need for his

2127   cohesion species concept as an alternative to reproductive isolation under the biological species

2128   concept to recognize species that interbreed but evolve as independent evolutionary lineages with

2129   distinct behaviors, ecologies, and morphologies. Others (e.g., Coyne and Orr 2004) adhere to

2130   reproductive isolation as a criterion, but advocate a detailed analysis of reproductive barriers

2131   without demanding absolute isolation.

2132   The existence of such a broad hybrid zone, particularly in the western Great Lakes states,

2133   indicates that reproductive isolation is incomplete. Indications of backcrosses between the two

2134   wolves have been reported (Koblmüller et al. 2009a, Fain et al. 2010), and may indicate some

2135   degree of past or ongoing breakdown in reproductive isolation. At this time, this wolf population

2136   remains heterogeneous with respect to the contributions of the mtDNA-Y-chromosome

2137   haplotype combinations or microsatellite DNA of these two species. Members of the gray wolf

2138   and eastern wolf lineages have probably been in contact since gray wolves first appeared in

2139   North America in the late Irvingtonian Land Mammal Age about 500,000 years ago (Kurtén and

2140   Anderson 1980). Despite a long history of contact with gray wolf and near-extirpation, the

2141   distinctive genetic markers of the eastern wolf persist. Whether the rate of hybridization and

2142   backcrossing is increasing or stable, which would indicate whether or the degree to which

2143   reproductive isolation may be deteriorating, is unknown. Conspecific combinations of mtDNA

2144   and Y-chromosome haplotypes are more common in male wolves of the western Great Lakes

2145   region than expected by random mating (Wheeldon et al. 2010), which suggests some constraint

2146   on admixture. Without this detailed information on the fitness and reproductive success of

2147   hybrids, the population relationships of the two species and whether they are stable or tending

2148   toward the complete merging of gene pools cannot be determined.

2149   The recently published analysis of SNP variation in *Canis* (vonHoldt et al. 2011) does not

2150   alter our interpretation of gray wolf, eastern wolf, and their interaction in the Great Lakes region.

2151   Difficulties with applying that study to questions concerning the taxonomic identity of Great

2152   Lakes wolves were previously noted. The broad-brush approach of vanHoldt et al. (2011)

2153   provides a valuable world-wide perspective on variation in *Canis*, but the taxonomic status of

2154   wolves in the Great Lakes region requires a finer-scale analysis that explores the interactions

2155   among individuals and packs on a more detailed geographic scale (Schwartz and Vucetich 2010).

2156   SNP analysis can contribute to our understanding if applied at this scale and integrated with

2157   information on mtDNA and Y-chromosome haplotypes of individual wolves. For example, the

2158   SNP composition of males with both eastern wolf mtDNA and Y-chromosome haplotypes can be

2159   compared with males with both gray wolf mtDNA and Y-chromosome haplotypes. SNP analysis

2160   can be applied at a local scale to reveal a detailed understanding of important features of

2161   interspecific hybridization (Fitzpatrick et al. 2009).

2162   Both natural and human-caused habitat changes have been implicated in other cases of

2163   inter-specific hybridization (Mayr 1963, p. 128; Seehausen et al. 1997). Habitat degradation

2164   together with human transport of individuals of one species into the range of another has resulted

2165   in breakdown in species integrity of the American black duck (*Anas rubripes*) through

2166   introgression from mallards (*Anas platyrhynchos*) (Mank et al. 2004), and the taxonomic

2167   integrity of some populations of California tiger salamanders (*Ambystoma californiense*) has

2168   been compromised by introgression from introduced barred tiger salamanders (*Ambystoma*

2169   *tigrinum*) (Fitzpatrick et al. 2009). The process by which previously isolated and divergent

2170   species experience introgressive hybridization has recently been called "reverse speciation" and

2171   identified as a concern in conservation (Seehausen 2006, Hendry 2009). Grant and Grant (2006)

2172   have termed as "despeciation" the process by which species of Galápagos Islands ground finches

2173   have lost morphological diagnosability through introgressive hybridization. They also suggest

2174   that, as environmental conditions change, reproductive isolation may be strengthened and

2175   diagnosability restored in a process they call merge-and-diverge dynamics.

2176   Because the essential features of the hybridization process for *C. lycaon* and *C. lupus* are

2177   unknown, it is unknown whether reverse speciation or despeciation is occurring. If populations

2178   expand and ecological conditions improve, there could even be a restoration or strengthening of

2179   isolating mechanisms. If isolating mechanisms deteriorate, it raises the question of at what point

2180   the process of despeciation is considered complete and only one species should be recognized. A

2181   precedent for formal taxonomic merging as a result of hybridization is the inclusion of the

2182   Mexican duck (*Anas diazi*) with mallards (*Anas platyrhynchos*) by the American Ornithologists'

2183   Union (1983), although that action has been questioned by the authors of a later mtDNA study of

2184   the mallard complex (McCracken et al. 2001). The possibility of merge-and-diverge dynamics

2185   (Grant and Grant 2006) suggests that a taxon should be recognized as long as individuals or

2186   populations representing its genetic distinctness remain. The long period of persistence of

2187 distinctive eastern wolf characteristics despite long contact with both *C. lupus* and coyotes

2188 further argues for caution.

2189 In comments on the study of Koblmüller et al. (2009a), Cronin and Mech (2009) state

2190 that taxonomy is subjective at and below the species level and propose the alternative of simply

2191 referring to the Great Lakes wolves as a population of mixed ancestry. "Mixed ancestry"

2192 encompasses diverse situations ranging from mild introgression to completely merged and

2193 homogenized populations, so that descriptor is too imprecise to characterize eastern wolves with

2194 the currently available information. We agree with Koblmüller et al. (2009b) that description of

2195 hybridization and introgression in the wolf population in the Great Lakes region does not

2196 preclude the consideration and recognition of either taxa or ecotypes, and that important

2197 information can be lost if taxonomic and ecological contexts are not considered.

2198

2199 **Species limits of *Canis lupus* with respect to the red wolf**

2200 Goldman's (1937) recognition of the red wolf as a distinct species (*Canis rufus*) has been

2201 followed by most taxonomic authorities, but the account for the red wolf in Wilson and Reeder

2202 (2005) accepts the conclusion of Wayne and Jenks (1991) that the red wolf is a hybrid and

2203 suggests that it should be considered of uncertain taxonomic status. As a compromise, it

2204 identifies the red wolf as a subspecies of *C. lupus*. The taxonomic status of the red wolf with

2205 respect to *C. lupus* will be evaluated in this section; its taxonomic relationship to the eastern wolf

2206 will be considered in the following section.

2207 On the basis of several discriminant function analyses, Nowak (1979) found the red wolf

2208 to be intermediate between coyotes and gray wolves. His series of skulls from before 1930 show

2209 no overlap with coyotes, except in central Texas. Series taken after 1930 show increasing

2210   amounts of overlap with coyotes as hybridization progressed, which he attributed to reduced

2211   densities of red wolves as a result of human control efforts.

2212   Red wolves from the captive population share all microsatellite DNA alleles with

2213   coyotes, and 8% of their alleles are not found in gray wolves (Roy et al. 1994). Red wolves are

2214   closer to coyotes than to gray wolves in multidimensional scaling analysis (Roy et al. 1994,

2215   1996, Figure 7 of this paper) and in neighbor-joining trees (Roy et al. 1994, 1996, Wilson et al.

2216   2000) based on genetic distances. Eleven additional historical red wolf samples (pre-1940)

2217   extended these results (Roy et al. 1996). Unique alleles were found in red wolves, but they were

2218   few compared to populations of coyotes and gray wolves.

2219   Two subsequent analyses of red wolf microsatellite DNA data from Roy et al. (1994,

2220   1996) in addition to expanded coyote and gray wolf samples came to different conclusions.

2221   Reich et al. (1999) accept the premise that the red wolf originated from hybridization between

2222   coyotes and gray wolves and estimate that the event occurred as much as 12,800 years ago, but

2223   probably within the last 2500 years. Bertorelle and Excoffier (1998) found these data compatible

2224   with a model of the red wolf and coyote as sister species that diverged much more recently than

2225   their separation from the gray wolf lineage. Accordingly, red wolves are also closer to coyotes

2226   than gray wolves at the major histocompatibility complex (MHC) gene *DRB*1 (Hedrick et al.

2227   2002).

2228   SNP data (vonHoldt et al. 2011) also place red wolf near coyotes and separate from

2229   wolves in the principal components analysis and in the STRUCTURE analysis at K = 3, which

2230   identifies three groups: wolves, dogs, and coyote combined with red wolf. F_{ST} ranged 0.08 to

2231   0.1 between red wolf and coyote and 0.12 to 0.18 between red wolf and western and Mexican

2232   gray wolves. SABER analysis assigned about 75 to 80 % of the red wolf genome to coyotes.

2233   The early restriction site data from mtDNA (Wayne and Jenks 1991) are difficult to

2234 interpret because samples were from canids captured while hybridization between red wolves

2235 and coyotes was underway, and individuals were classified on morphological criteria as red

2236 wolves, coyotes, or hybrids. The founders of the captive population were found to have the same

2237 haplotype as two coyotes from Louisiana. Mitochondrial cytochrome *b* gene haplotypes from six

2238 pre-1930 skins identified as from red wolves and reference samples of gray wolves and coyotes

2239 were distributed among two clades, corresponding to a wolf clade and a coyote clade (Wayne

2240 and Jenks 1991). The six historic red wolves were distributed evenly among the two clades. The

2241 three historic specimens in the coyote clade were not identical to any coyote, and one of three

2242 historic red wolves in the wolf clade had the same haplotype as a gray wolf. Eleven additional

2243 historic samples (pre-1940) extended these results (Roy et al. 1996). Eight historical samples

2244 were placed in the coyote clade in a parsimony tree, but none were identical to coyotes. One of

2245 three individuals in the gray wolf clade was identical to a gray wolf haplotype.

2246   The mtDNA control region sequence of the red wolf captive population is unique and has

2247 an average divergence from coyotes of 3.24%, compared to an average of 2.79% divergence

2248 among coyote haplotypes (Adams et al. 2003). The red wolf is in the coyote clade of the

2249 neighbor-joining tree of haplotypes in this study.

2250   Hailer and Leonard's (2008) study of mtDNA control region and Y-chromosome

2251 haplotypes explored potential reproductive isolation among the Mexican wolf (*C. l. baileyi*), red

2252 wolf, and coyotes, which were historically sympatric in central Texas. The single control region

2253 haplotype found in the captive population of red wolves was in the coyote clade (two base pairs

2254 different from the nearest coyote), which was strongly divergent from the wolf clade, including

2255 Mexican wolf. Of the five red wolves whose Y-chromosome haplotypes were identified, four

2256   shared a haplotype with coyotes, while one was identical to domestic dog (Bannasch et al. 2005).

2257   Inter-species hybridization has had an influence on these three species, but the greatest effect

2258   was on the red wolf, especially on its Y-chromosome ancestry (Hailer and Leonard 2008).

2259   Several aspects of the available data hinder clear inferences about taxonomic

2260   comparisons of the red wolf and gray wolves. The data are derived from a relatively small

2261   number of historical specimens, and from captive populations of red wolf and Mexican wolves

2262   that were derived from very small numbers of founders that are not likely to be representative of

2263   historical populations. Founder effects and genetic drift have likely affected the genetic

2264   composition of the captive populations. Further, it is difficult to separate the results of rare, and

2265   old incidences of hybridization from the undoubted introgression from coyotes that was

2266   occurring at the time the last wild red wolves were rescued (Nowak 1979).

2267   Even with these limitations, genetic information places most individuals of the red wolf

2268   closer to coyotes than to gray wolves. This is clear in the nuclear microsatellite DNA data

2269   despite the sharing of many alleles among species and relatively few private alleles in red

2270   wolves. Some mtDNA cytochrome *b* gene haplotypes from historical red wolf samples are wolf-

2271   like, but the red wolf haplotype from the higher-resolution mtDNA control region of red wolves

2272   in the recovery program is unique and in the coyote clade. The Y-chromosome haplotypes from

2273   red wolves in the recovery program indicates introgression from coyotes (haplotype H15) and

2274   from domestic dog (haplotype H1, Table 5). The morphometric analyses of chronological series

2275   of *Canis* by Nowak (1979) documents the historical existence of a morphologically identifiable

2276   red wolf, followed by the decline in its morphological distinctiveness as hybridization with

2277   coyotes progressed. There is very limited information from historical specimens (Wayne and

2278   Jenks 1991, Roy et al. 1996), so we do not know the genetic composition of that historical red

2279   wolf, but most red wolf individuals for which we have genetic information are closer to coyotes

2280   rather than to gray wolves. The closer relationship of the red wolf to coyotes than to gray wolves

2281   indicates it is outside of the gray wolf lineage and is not within the species limits of *Canis lupus*.

2282   It does raise the question of whether the red wolf is within the species limits of any members of

2283   the greater coyote clade, including the eastern wolf, and this will be evaluated in the next section.

2284

2285   **Species limits within the coyote clade**

2286   The mtDNA haplotypes of red wolf and some eastern wolves are part of a greater

2287   mtDNA clade that includes coyote haplotypes. This section evaluates whether red wolf and

2288   eastern wolf are within the species limits of coyotes.

2289

2290   *Nuclear genetic markers.* Red wolf (both from captive population and historical specimens) and

2291   eastern wolf are outside the 95% confidence ellipse encompassing coyotes based on multi-

2292   dimensional scaling analysis of 10 microsatellite loci (Roy et al. 1996, Figure 7 of this paper). A

2293   southern Quebec sample, which likely has some representation of eastern wolf, was even more

2294   distant from coyotes towards gray wolves. This pattern of divergence of red wolf and eastern

2295   wolf from coyotes is evident in a neighbor-joining tree based on genetic distances (*D* of Nei

2296   1978) from the same data. The distances between eastern wolves and coyotes (0.216 and 0.341

2297   for historical and captive red wolves, respectively) were greater than the average distances

2298   among coyote samples (0.188) (Roy et al. 1996).

2299   Analysis of microsatellite DNA data from coyote, eastern wolf, and red wolf using

2300   neighbor-joining trees, log-likelihood analysis, and probability-of-identity analysis by Wilson et

2301   al. (2000) distinguish these groups from gray wolves, and also distinguish eastern wolf and red

2302   wolf from coyotes. Additional information comparing red wolves and coyotes is provided by

2303   gene sequences in the major histocompatibility locus (MHC) (Hedrick et al. 2002).   Three of

2304   four red wolf alleles were also found in coyotes from Texas and North Carolina. The fourth

2305   allele was a single nucleotide different from an allele found in coyotes.

2306   STRUCTURE analyses have consistently identified the eastern wolf as a discrete group.

2307   The analysis by Wilson et al. (2009) separated Algonquin eastern wolf from the nearby

2308   population of "Tweed wolves" of the Frontenac Axis, which are primarily coyote, and from

2309   Texas coyotes as well. STRUCTURE and GENELAND analyses by Rutledge et al. (2010b)

2310   indicate that despite interbreeding, the Algonquin wolves remain genetically distinct from the

2311   Frontenac Axis population. Rutledge et al. (2010b) also reported a tendency of conspecific

2312   mating at Algonquin Provincial Park, which can contribute to the reinforcement of reproductive

2313   isolation. Other STRUCTURE analyses identified an eastern wolf (or Great Lakes wolf) cluster

2314   in comparisons with western coyotes and eastern coyotes (Koblmüller et al. 2009a), and western

2315   Great Lakes states clusters in analyses with sympatric coyotes (Fain et al. 2010, Wheeldon et al.

2316   2010).

2317   Comparison of genetic distance measures reported in these studies further support genetic

2318   distinctiveness of the eastern wolf. F_{ST} values between eastern wolves from Algonquin

2319   Provincial Park and coyotes are greater than between Algonquin and nearby hybridized eastern

2320   wolf-gray wolf populations (Grewal et al. 2004), and F_{ST} is high between western Great Lakes

2321   states wolves and sympatric coyotes. Genetic distance (as measured by Θ) is greater between

2322   eastern wolf (Great Lakes wolf) and coyotes than between eastern wolf and gray wolves

2323   (Koblmüller et al. 2009a, Table 6 of this paper). A factorial correspondence analysis of these

2324   data indicates that eastern and western coyotes are much closer to one another than either is to

2325   the great majority of eastern wolves (Figure 3(d) of Koblmüller et al., 2009a).

2326   These data on nuclear genetic markers consistently portray a pattern indicating coyote,

2327   eastern wolf, and red wolf are related, but with the red wolf and eastern wolf as outliers to

2328   coyote.

2329   Analyses of SNP data (vonHoldt et al. 2011) generally indicate that red wolf and eastern

2330   wolf are both more divergent from coyotes than coyote populations are from one another. The

2331   principal components analysis places the red wolf as a discrete cluster near coyotes, although the

2332   genetic bottleneck associated with the founding of the red wolf captive population has very likely

2333   contributed to this discreteness. Eastern wolf is more divergent from coyote. The possible

2334   inclusion gray wolf individuals or introgression of gray wolf into the eastern wolf population

2335   may have contributed to this divergence; however, the two individuals from Algonquin

2336   Provincial Park, where mtDNA and Y-chromosome haplotypes indicate only minimal gray wolf

2337   influence and coyote introgression is evident (Rutledge et al. 2010b), are near other eastern

2338   wolves and divergent from coyote.

2339   STRUCTURE analysis of the SNP data (vonHoldt et al. 2011) separates eastern wolf

2340   (together with western wolves) from coyote and dog at K = 3. Red wolf is not separated from

2341   coyote until K = 9. F_{ST} among the three groupings of the coyote sample (western,

2342   midwestern/southern, and northeastern) range 0.2 to 0.5. Both red wolf and eastern wolf are

2343   more divergent from coyotes: F_{ST} between red wolf and coyotes ranges 0.08 to 0.1, and between

2344   eastern wolf and coyotes ranges 0.08 to 0.11.

2345

2346   *Mitochondrial DNA and Y-chromosome haplotypes.* Mitochondrial cytochrome *b* gene

2347   sequences are ambiguous with respect to red wolf-coyote divergence, as discussed earlier, with

2348   three of 11 historical specimens distributed in the gray wolf clade (Wayne and Jenks 1991, Roy

2349   et al. 1996). The three red wolf haplotypes in the coyote cytochrome *b* clade are distributed on

2350   different branches. The single control region haplotype from the captive breeding program for

2351   the red wolf appears within the coyote clade in neighbor-joining trees from relatively basal (Vila

2352   et al 1999, Figure 1; Wilson et al. 2000, Figure 5; Figures 10 and 11 of this paper; Wilson et al.

2353   2003; Hailer and Leonard 2008) to embedded among various coyote clades (Adams et al 2003,

2354   Figure 2; Fain et al. 2010, Figure 3). The basal positions are generally not well supported.

2355   In their study designed specifically to examine coyote and red wolf in a former area of

2356   sympatry, Hailer and Leonard (2008) found that the single red wolf control region haplotype

2357   from the captive population was not shared with any coyote, but the observed high haplotype

2358   diversity of coyotes suggests that additional coyote haplotypes may remain unsampled. It is

2359   significant that red wolf and coyote in a former area of sympatry and with documented modern

2360   hybridization do not share mtDNA haplotypes. As described earlier, the two Y-chromosome

2361   haplotypes found in red wolves in the recovery program, reflect coyote and dog introgression

2362   (Table 5 of this paper).

2363   The early restriction site analysis of mtDNA of wolves from the Great Lakes region

2364   found haplotypes distributed among wolf and coyote clades (Lehman et al. 1991, Figure 3).

2365   Minimum spanning and neighbor-joining trees of mtDNA control region sequences group unique

2366   eastern wolf haplotypes C1 and C3 together basal to coyotes, but another eastern wolf haplotype,

2367   C13, while not identical to any coyote haplotype, is embedded with coyote haplotypes (Wilson et

2368   al. 2000, Figure 5; Figures 10 and 11 of this paper; Fain et al. 2010).

2369   Additional mtDNA control region sequences from historical wolf specimens from the

2370   Great Lakes area confirm this pattern (Leonard and Wayne 2008). Koblmüller et al. (2009a)

2371   report sharing of haplotypes between coyotes and eastern wolves, but information is not provided

2372   on the geographical source of these individuals.

2373   Analysis of mtDNA and Y-chromosome haplotypes from *Canis* of the western Great

2374   Lakes states (Fain et al. 2010, Wheeldon et al. 2010) indicates no sharing of haplotypes between

2375   eastern wolves and coyotes, although phylogenetic analysis indicates that there may have been

2376   past rare incidences of hybridization resulting in a low level of wolf influence on coyotes.

2377   Koblmüller et al. (2009a) reported that sharing of Y-chromosome haplotypes by eastern wolves

2378   and coyotes is rare.

2379   Hybridization between eastern wolves and coyotes in the eastern Great Lakes regions has

2380   primarily affected eastern coyotes, including those of the northeastern United States (Kays et al.

2381   2009), and the "Tweed wolf" of southern Ontario, which Wilson et al. (2009) consider a

2382   population of eastern coyote. In a recent study (Rutledge 2010b) coyote mtDNA was found in

2383   wolves in Algonquin Provincial Park, but shared coyote-eastern wolf Y-chromosome haplotypes

2384   were less common. Eastern wolf Y-chromosome haplotypes were more frequent in breeding

2385   males than predicted by random mating, so assortative mating may be playing a role in

2386   maintaining a cohesive eastern wolf population despite past interbreeding with gray wolves and

2387   coyotes.

2388   The magnitude of divergence between red wolf and eastern wolf clades and other

2389   mtDNA clades within the greater coyote clade can be evaluated by comparison to divergence

2390   among clades strictly attributed to coyotes. Red wolf-coyote and eastern wolf-coyote

2391   divergences are generally greater that coyote-coyote divergences (Table 9). Mitochondrial DNA

2392 cytochrome *b* sequences within the coyote clade had an average sequence divergence from

2393 coyotes of 1.2%, compared to an average of 1.1% among coyote haplotypes (Wayne and Jenks

2394 1991, Roy et al. 1996, Table 9 of this paper). For mtDNA control region haplotypes, the average

2395 divergence between C2 of red wolf and coyotes is 3.2%, compared to an average of 2.8% among

2396 coyote haplotypes.

2397

2398 *Conclusions.* The eastern wolf forms a divergent clade basal to the greater coyote clade for both

2399 mtDNA and Y-chromosome haplotypes (Wilson et al., 2000, Fain et al. 2010), with additional

2400 clades consistent with subsequent, ancient, introgression from coyotes. Generally, sequence

2401 divergences and branching patterns of the divergent clades indicate a deeper and older

2402 divergence between eastern wolf and coyote than among coyote branches of the greater coyote

2403 clade. This agrees with various nuclear microsatellite DNA studies including gray wolves and

2404 coyotes of the Great Lakes region that show eastern wolf as an identifiable cluster (Koblmüller et

2405 al. 2009a, Fain et al. 2010, Rutledge 2010b, Wheeldon et al. 2010) and an outlier to coyotes (Roy

2406 et al. 1996). Eastern wolves and coyotes do not interbreed where they are both sympatric and

2407 abundant in the western Great Lakes states (Fain et al. 2010, Wheeldon et al., 2010). In the

2408 eastern portion of their range, eastern wolves have experienced some coyote introgression

2409 through the maternal line as indicated by mtDNA, but appear to be maintaining a cohesive

2410 population in Algonquin Provincial Park assisted by a preference for intraspecific mating

2411 (Rutledge et al. 2010b). Introgression from coyotes through the maternal line likely occurred

2412 historically when the eastern wolf population was at lower densities and the availability of

2413 conspecific mates was reduced. Reproductive isolation may not be complete in this eastern

2414 portion of the eastern wolf range, but the eastern wolf is displaying cohesion and maintaining

2415   itself as a phylogenetically unique lineage in the Great Lakes region distinct from sympatric

2416   coyotes.

2417   Red wolf mtDNA also forms a divergent clade within the greater coyote clade. This is

2418   consistent with microsatellite DNA data that indicate it as an outlier to coyotes. Its Y-

2419   chromosome haplotypes do not form a distinct clade, the H15 haplotype is identical to a Texas

2420   coyote haplotype, whereas the H1 haplotype is identical to domestic dog haplotypes (Bannasch

2421   et al. 2005). As a result, the Y-chromosome genetics of red wolves is not useful for phylogenetic

2422   inference. A morphologically discrete and identifiable red wolf has survived reduction in its

2423   population size and range and subsequent hybridization with coyotes and either dogs or wolf-dog

2424   hybrids (Nowak 1979). The modern descendents of that original red wolf population show the

2425   influence of hybridization as the population status of the red wolf declined. The red wolf

2426   remains an identifiable lineage separate from coyotes, despite past coyote hybridization and the

2427   continuing threat from coyotes in areas where the red wolf has been re-introduced (Adams et al.

2428   2003).

2429   Red wolf and eastern wolf remain identifiable lineages that have evolved in North

2430   America with coyotes. Despite long histories of sympatry with or proximity to coyote

2431   populations and the ability of *Canis* species to interbreed, instances of ancient hybridization

2432   between these two lineages and coyotes have been rare. Major threats of introgression can be

2433   associated with recent reduction of wolf population densities, and alteration of natural habitat

2434   that once contributed to the isolation of the different lineages.

2435

2436   **Relationship of red wolf and eastern wolf**

2437   In the previous section, red wolf and eastern wolf were concluded to be lineages

2438   divergent from coyotes. This section considers whether red wolf and eastern wolf are close

2439   enough to be considered a single species under the older name of *Canis lycaon* as proposed by

2440   Wilson et al. (2000) based on their genetic similarities.

2441   Two very different hypotheses involving hybridization have been proposed to explain the

2442   general similarities between red wolf and eastern wolf. Nowak's (1979, 1995, 2002, 2003)

2443   morphometric analyses describe eastern wolf, which he considers to be a subspecies of gray

2444   wolf, as statistically intermediate between gray wolf and red wolf, with red wolf resembling

2445   eastern wolf more than it does any gray wolf. Nowak (2002) proposed that the eastern wolf may

2446   have originated as a result of hybridization between red wolf and gray wolf. This hypothesis is

2447   not supported by the available genetic data. Samples of wolves from Algonquin Provincial Park

2448   and surrounding area reported by Wilson et al. (2000, Figure 5A) have either the C1 haplotype

2449   unique to eastern wolf, or either coyote or coyote-like haplotypes, but no haplotypes associated

2450   with gray wolf to the north and west. A sample of three individuals from "north of Algonquin

2451   Park" (Wilson et al. 2000) included both the eastern wolf C1 haplotype and gray wolf haplotype

2452   lu32, which indicates interaction between the species further to the north. Subsequent studies

2453   have reported a low incidence of gray wolf mtDNA and Y-chromosome haplotypes in Algonquin

2454   Provincial Park (Grewal et al. 2004, Rutledge et al. 2010b). The park is within the range of

2455   eastern wolf as recognized by Nowak (2003), and therefore should include a substantially greater

2456   percentage of gray wolf genetic markers if hybridization involving that species was a major

2457   factor in the origin of the eastern wolf.

2458   Roy et al. (1994, 1996) attributed the genetic similarity between red wolf and eastern

2459   wolf to both having hybridized with coyotes. The mtDNA haplotypes initially identified as

2460   having been derived from coyotes (Lehman et al. 1991) are different in red wolf and eastern wolf

2461   and have not been found in western coyotes, the presumed source of hybridization (Roy et al.

2462   1996; Wilson et al. 2000, 2003; Hailer and Leonard 2008). A mtDNA haplotype is shared by

2463   eastern wolf and coyotes of the northeastern United States, but it is interpreted as an eastern wolf

2464   haplotype that has influenced this eastern coyote population and not the reverse (Kays et al.

2465   2009). As discussed in a previous section, the similarity to coyote mtDNA haplotypes is more

2466   likely the result of a shared evolutionary history with coyotes separate from gray wolves, with

2467   some rare instances of ancient introgression.

2468   The proposal of Wilson et al. (2000) that eastern wolf and red wolf may be the same

2469   species is based on similarities in nuclear microsatellite DNA loci and mtDNA haplotypes. Both

2470   sets of genetic markers indicate that there is genetic divergence between eastern wolf and red

2471   wolf. This divergence could represent coyote or dog introgression in red wolf, geographic

2472   variation within a single species, or it may indicate genetic discontinuity between two species.

2473   Interpretation of the taxonomic significance of this difference is confounded by the large

2474   geographic distance between the sources of samples: the red wolf samples are all from

2475   descendants of wolves captured in eastern Texas and southwestern Louisiana, while eastern wolf

2476   samples were collected at locations over 2000 km away in eastern Canada. There are no genetic

2477   samples from intervening areas, except for the historical samples from Maine and New York,

2478   which have eastern wolf mtDNA haplotypes (Wilson et al 2003). Nowak (2002) identifies a

2479   single historical skull from Maine as red wolf based on its morphological features. If these

2480   wolves are considered as belonging to two species, this gap in sampling would make it

2481   impossible to infer from existing data the past geographic ranges of the two putative species and

2482   any possible interactions between them.

2483   Red wolf and eastern wolf are more similar to each other at autosomal microsatellite loci

2484   than either is to Texas coyotes or western gray wolves (Roy et al. 1994, 1996; Wilson et al.

2485   2000). Microsatellite data have very limited value for this comparison because of the large

2486   geographic distances between the sources of the samples and the loss of linearity with distance of

2487   common distance measures for microsatellite DNA data (Paetkau et al. 1997). In addition,

2488   distances are based largely on allele frequency differences, which have undoubtedly been

2489   affected by severe population bottlenecks experienced as the red wolf population declined to

2490   near extinction before the founding of the captive red wolf population.

2491   Analyses of SNP data (vonHoldt et al. 2011) generally indicate divergence between red

2492   wolf and eastern wolf. As indicated in the previous section, principal components analysis

2493   places red wolf near coyotes and eastern wolf near gray wolves. STRUCTURE analyses

2494   similarly place red wolf with coyotes and eastern wolf with gray wolves at K =3. SABER

2495   analysis attributes more gray wolf ancestry to eastern wolf and more coyote ancestry to red wolf.

2496 F_{ST} values (vonHoldt et al. 2011, Supplemental Table S3) also indicate divergence between

2497   eastern wolf and red wolf. F_{ST} between red wolf and eastern wolf is reported as 0.11, which is

2498   comparable the divergence of each from coyotes. For comparison, F_{ST} values among coyotes in

2499   this study ranged 0.2 to 0.5. Limiting the interpretation of the SNP data are the small sample

2500   size of the eastern wolf sample (N = 19), and the differential influences of admixture on eastern

2501   wolf and red wolf: possible inclusion of or introgression from gray wolves in the western Great

2502   Lakes region, admixture of coyotes and eastern wolves in the eastern Great Lakes region, and

2503   admixture between coyote and red wolf.

2504   Mitochondrial DNA data could lend support to the proposal of Wilson et al. (2000) that

2505   eastern wolf and red wolf are one species if they were found to cluster together within the greater

2506   coyote clade. In the minimum spanning network of Wilson et al. (2000, Figure 5A) the

2507   haplotype of red wolf is closer to coyotes, but appears in a basal position on the branch leading

2508   to eastern wolf. In neighbor joining and maximum parsimony trees in analyses that included

2509   historical specimens (Wilson et al. 2003), red wolf was not basal to eastern wolf, but both

2510   formed separate branches that were basal to coyote haplotypes. Red wolf and eastern wolf did

2511   not cluster together in either a parsimony consensus cladogram (Leonard and Wayne 2008,

2512   Figure 1(b)) or neighbor-joining tree (Fain et al. 2010, Figure 3) of control region mtDNA data,

2513   where they were separated by intervening coyote clades.

2514   Red wolf and eastern wolf do not share comparable Y-chromosome haplotypes (Hailer

2515   and Leonard 2008, Fain et al. 2010, Rutledge et al. 2010b), but as discussed earlier, four of five

2516   red wolves shared a haplotype found in Texas coyotes and is likely the result of coyote

2517   introgression (Hailer and Leonard 2008) and the "gray wolf-like" haplotype reported by Hailer

2518   and Leonard (2008) is consistent with dog origin (Bannasch et al. 2005).   Y-chromosome

2519   composition of the captive red wolf population is likely the result of wolf-dog hybrid and coyote

2520   introgression and is therefore not comparable to eastern wolf haplotypes.

2521   The available information on haplotype composition and relationships indicate that

2522   eastern wolf and red wolf do not share mtDNA haplotypes and do not together form a single

2523   group exclusive of coyote lineages. Independent evolution of these two taxa from different

2524   lineages of coyote-clade ancestors is more consistent with the available genetic data and argues

2525   against combining them as a single species but retaining them as *Canis lycaon* and *Canis rufus*.

2526

2527   *Nomenclatural issues.* Regardless of present lack of genetic support for combining the taxa,

2528   some nomenclatural problems would need to be addressed before uniting *C. lycaon* and *C. rufus*.

2529   A compromise approach would be to recognize *C. lycaon* and *C. rufus* as subspecies within the

2530   same species. This would require the formal publication of new name combinations for at least

2531   one subspecies. In addition, there is significant geographic variation in genetic composition

2532   within a more broadly defined taxon that includes both *C. lycaon* and *C. rufus*. Moreover,

2533   Texas wolves occupied a very different environment than wolves in eastern Canada.

2534   The appropriate name for a single species that would encompass both *C. lycaon* and *C.*

2535   *rufus* remains an issue. Wilson et al. (2000) suggested that the name be *C. lycaon*. The rule of

2536   chronological priority normally applies in such cases, and the name *Canis lycaon* was published

2537   earlier than *Canis lupus* var. *rufus* (Table 1). Two reviewers of an earlier draft of this paper

2538   questioned the applicability of the older name in this case owing to uncertainty about the identity

2539   of the type specimen of *Canis lycaon* because it may have been a hybrid. As explained by

2540   Goldman (1944), the type is the individual portrayed in Schreber's illustration, which was in turn

2541   based on a figure published by Buffon in 1761. Article 73.1.4 of the International Code for

2542   Zoological Nomenclature (1999) provides for such instances: "Designation of an illustration of a

2543   single specimen as a holotype is to be treated as designation of the specimen illustrated; the fact

2544   that the specimen no longer exists or cannot be traced does not of itself invalidate the

2545   designation." Because the disposition of the remains of the illustrated specimen is unknown, and

2546   the holotype must be of the same group to which the species name is applied, the identity of the

2547   specimen portrayed is important.

2548   The type locality was restricted by Goldman (1937) to the vicinity of Quebec, Quebec.

2549   Wolves in this region may have already been interbreeding with coyotes or dogs at the time the

2550   holotype was collected. Support for earlier hybridization is provided by the presence of either

2551   coyote or dog mtDNA in wolf (based on morphology) remains of four individuals from a 400-

2552   500-year-old archaeological site in southern Ontario (Rutledge et al. 2010a). Article 23.8 of the

2553   International Code specifies that, "a species-group name established for an animal later found to

2554   be a hybrid must not be used as the valid name of either of the parental species, even if it is older

2555   than all other available names for them." Pocock's (1935) argument that the type may have been

2556   a hybrid was rejected by Goldman (1937), partially on the basis that a hybrid could still serve as

2557   the type, which is in conflict with today's Code.

2558   The next oldest name in Goldman's (1944) synonymy for *C. lycaon* is *Canis lupus*

2559   *canadensis* (Table 1). Allen and Barbour (1937) note that the type specimen for *C. l. canadensis*

2560   is a skull illustrated by Plainville and that the locality was given only as Canada, so questions

2561   may also be raised about its identity and relation to modern wolf populations in eastern Canada.

2562   In this case the holotype of *C. rufus* (type locality: Austin, Texas) might be more appropriate

2563   because hybridization with coyotes would have been unlikely at the time it was described.

2564   Nowak (2009) believes that the holotype of *C. lycaon* is actually a specimen of *C. rufus* based in

2565   its description as black, which he describes as a well-known coat color in *C. rufus*, but unusual

2566   for *C. lycaon*. Black coat color could also indicate that the individual had dog ancestry. Current

2567   wolves nearest the *C. lycaon* type locality (vicinity of Quebec), however, have the mtDNA

2568   haplotypes of *C. lycaon.* Even if additional evidence should provide support for formally

2569   combining these taxa, this issue would need to be resolved before formal changes in taxonomy

2570   are made.

2571

2572   **The subspecies of *Canis lupus***

2573   The following analysis and discussion is organized by the remaining (less *C. l. lycaon*)

2574   subspecies of *C. lupus* recognized by Nowak (1995). This does not mean that Nowak's

2575 classification is accepted without consideration of alternative classifications. The analysis

2576 therefore includes consideration of formerly-recognized subspecies (e. g., Goldman [1944], Hall

2577 [1981]) that were reduced to synonymy by Nowak (1995) where patterns of variation within

2578 these four subspecies suggest that some finer-scale taxonomic subdivision might be

2579 recognizable.

2580

2581 *Canis lupus baileyi (Mexican wolf).* Both morphometric and genetic evidence support the

2582 distinctiveness of *C. l. baileyi* and its recognition as a subspecies. Genetic analysis of living

2583 specimens is limited to the descendents of the founders of the captive breeding population,

2584 thought to be seven individuals (Hedrick et al. 1997). Although the effects of genetic drift and a

2585 small founder population has likely increased the observed divergence of living *C. l. baileyi* from

2586 other wolves at autosomal microsatellite DNA loci (García-Moreno et al. 1996), they cannot

2587 account for the unique mtDNA haplotype (Roy et al. 1996, Vilà et al. 1999) and several private

2588 microsatellite DNA alleles (García-Moreno et al. 1996) found in *C. l. baileyi*. Additional genetic

2589 data from historical, museum specimens (Leonard et al. 2005) have corroborated the results

2590 obtained from living individuals, and further indicate that the "southern mtDNA clade" of the

2591 Mexican wolf is divergent from other North American wolves.

2592 Comparisons of mtDNA sequence divergences among *C. lupus* haplotypes support

2593 recognition of *C. l. baileyi* as a subspecies rather than as a species distinct from other *C. lupus*.

2594 The predominant *C. l. baileyi* haplotype has a sequence divergence of 2.2% from the closest

2595 other North American *C. lupus* haplotype (Wayne and Vilà 2003, p. 228), compared to sequence

2596 divergences averaging 2.9% within *C. lupus* (Vilà et al. 1999, p. 2093), 8% between *C. lupus* and

2597 either *C. lycaon* or *C. rufus*, and 10% between gray wolf and coyote (Wilson et al. 2000, p.

2598 2159).

2599 Analyses of SNP data (vonHoldt et al. 2011) from 10 Mexican wolves from the captive

2600 breeding program also indicate the distinctness of the Mexican wolf. Principal components

2601 analysis of the North American gray wolves (vonHoldt et al. 2011, Supplementary Figure S2)

2602 separates Mexican wolf from other gray wolves on the first principal components axis, which

2603 accounts for 6.6% of the variance. Mexican wolf is basal to other North American gray wolves

2604 in both a neighbor-joining tree and a phylogram (vonHoldt et al. 2011, Supplementary Figure

2605 S5). F_{ST} between Mexican wolf and other western gray wolves is 0.1, which is greater than F_{ST}

2606 values, which range 0.01 to 0.08 among western gray wolves from different regions. While

2607 these results are consistent with other genetic data, a founder effect in establishing the captive

2608 Mexican wolf population may have contributed to the measures of divergence based on both

2609 SNPs and microsatellite DNA.

2610 There is consensus on the valid taxonomic standing of *C. l. baileyi*, but there is some

2611 controversy based on interpretation of morphometric data on the historical boundaries of the

2612 subspecies. Nowak (1995) recognized *C. l. baileyi* as a subspecies, but did not adopt Bogan and

2613 Mehlhop's (1983) inclusion of *C. l. mogollonensis* and *C. l. monstrabilis* as its synonyms. These

2614 different interpretations may be related to larger sample sizes used by Bogan and Mehlhop

2615 (1983), who studied 253 skulls of the three subspecies in question, compared to 88 skulls studied

2616 by Nowak (1995). It may also be related to Bogan and Mehlhop's (1983, p. 15) preference for

2617 principal components analysis as a more objective method for assessing overlap in characters

2618 than discriminant function analysis, which was used by Nowak (1979, p. 4). Bogan and

2619 Mehlhop (1983) also carried out discriminant function analyses on their data and found

2620   intermediacy of skulls assigned to *C. l. mogollonensis* between *C. l. baileyi* and more northern

2621   wolves. The two different discriminant function analyses have generally comparable outcomes,

2622   so the difference is in interpreting which subspecies to assign a collection of individuals that is

2623   intermediate between recognized taxa. Bogan and Mehlhop (1983) and Nowak (1995) agree that

2624   the range of *C. l. mogollonensis* in Arizona was a transition zone where *C. l. baileyi* intergraded

2625   with more northern *C. lupus*, which is consistent with the limited available genetic data from

2626   historical specimens (Leonard et al. 2005). Wolves were long ago extirpated — perhaps by the

2627   1940s (Parsons 1996) — within the ranges of *C. l. monstrabilis* and *C. l. mogollonensis* so the

2628   controversy is now primarily of historical interest.

2629   The phylogenetically closer relationship of *C. l. baileyi* to certain Eurasian wolf

2630   populations than to other North American *C. lupus* (Vilà et al. 1999, Wayne and Vilà 2003)

2631   indicates that contact was secondary between *C. l. baileyi*, as delineated by Bogan and Mehlhop

2632   (1983), and the later-arriving, more northerly *C. l. nubilus*. Both morphometric (Nowak 1995, p.

2633   385) and genetic data (Leonard et al. 2005) are consistent in indicating that, once *C. l. baileyi*

2634   came into contact with more recent *C. lupus* invaders from Eurasia, there was a broad area of

2635   reproductive interaction between them. This interaction could have been in the form of a

2636   relatively stable and broad zone of intergradation between them, or *C. l. nubilus* could have

2637   incorporated genetic elements of *C. l. baileyi* as it rapidly displaced the latter subspecies to the

2638   south. General models on plants and animals have demonstrated the process by which local

2639   genes are incorporated into an invading population (Currant et al. 2008). The interaction has

2640   been described more locally in Arizona by the morphometric data (Bogan and Mehlhop 1983,

2641   Nowak 1985), and more expansively by the mtDNA data from historical specimens, where a

2642   northern haplotype associated with *C. l. nubilus* was found in Arizona and southern haplotypes

2643   associated with *C. l. baileyi* were found as far north as Nebraska.

2644   *Canis lupus baileyi* and *Canis rufus* do not overlap in morphometric variation of skull

2645   features (Nowak 1979). The genetic data, particularly that of Hailer and Leonard (2008),

2646   indicate that if hybridization has occurred between these species it has apparently not affected

2647   the genetic composition of *C. l. baileyi*, with one exception. The Y-chromosome haplotype H29

2648   that Hailer and Leonard (2008, Fig 3B) found in some *C. l. baileyi* and identified as a wolf

2649   haplotype is common in dogs (Table 5). The *C. l. baileyi* with H29 are all descended from an

2650   individual in the "Aragon lineage," which has a nuclear DNA composition (based on

2651   microsatellites) that clusters with other *C. l. baileyi* (Hedrick et al. 1997).

2652

2653   *Canis lupus arctos (Arctic wolf).* The three high Arctic Islands sampling areas for *C. l. arctos*

2654   were grouped together in a neighbor-joining distance analysis based on microsatellite DNA data

2655   (Carmichael et al. 2008, Figure 3(B)), but the authors observed that the island populations

2656   exhibited only one private allele, and that their unpublished mtDNA data did not identify any

2657   unique Arctic Islands haplotypes. Based on the assumption that a long isolation in an Arctic

2658   refugium, as proposed by Nowak (1983), should have resulted in a higher proportion of unique

2659   alleles, Carmichael et al. (2008) concluded that these Arctic Islands populations are the result of

2660   recent colonization from the mainland. Their interpretation was further supported by low levels

2661   of genetic diversity in the island wolves. Low levels of diversity also affect the reliability of

2662   distance measures calculated from microsatellite loci (Paetkau et al. 1997). The genetic

2663   differences observed under these circumstances are not likely to be of taxonomic significance.

2664   The morphometric data in support of recognition of *C. l. arctos* have limitations. The

2665   overlap with mainland subspecies (*C. l. nubilus* and *C. l. occidentalis*) is not minimal (Nowak

2666   1995, Figure 9), and the large polygons representing the mainland subspecies are likely affected

2667   by the very large scale of geographic sampling of the mainland subspecies. A more relevant

2668   comparison for evaluating taxonomically significant discontinuity between island and mainland

2669   populations would be between island and adjacent, coastal-mainland populations. Coastal-

2670   mainland populations do not appear to be well-represented in either morphometric study (Nowak

2671   1995, Mulders 1997).

2672   The genetic data together with difficulties in interpreting the morphometric data do not

2673   provide clear support for subspecies recognition of *C. l. arctos*. This conclusion is tentative

2674   because it is based on lack of supportive data rather than definitive information that these

2675   populations are not taxonomically recognizable. The genetic data consist only of autosomal

2676   microsatellite DNA, and some preliminary mtDNA data that did not detect unique haplotypes in

2677   the island populations (Carmichael 2008, p. 885). Y-chromosome and additional mtDNA data

2678   could better resolve the relationship between island and mainland populations, and therefore the

2679   taxonomic standing of *C. l. arctos*.

2680

2681   *Canis lupus occidentalis (northern timber wolf).* When viewed in the context of Nowak's (1995)

2682   subspecies classification, Skeel and Carbyn's (1977) morphometric analysis indicates average

2683   differences between *C. l. occidentalis* and *C. l. nubilus* with some overlap.

2684   Mitochondrial DNA haplotypes based on restriction site mapping show the same

2685   haplotypes from Alaska to Montana and Minnesota; these samples were mostly within the range

2686   of *C. l. occidentalis* (Wayne et al. 1992, 1995). An exception is a haplotype that was unique to

2687   Manitoba, likely within the range of *C. l. nubilus*. These studies do not show strong

2688   differentiation between *C. l. occidentalis* and *C. l. nubilus*, but the samples were used for a

2689   general, world-wide review of wolf variation and are therefore small and widespread and not at a

2690   suitable scale for resolving potential subspecies differences. A similar pattern (in this case, a

2691   lack of geographic pattern) with respect to resolving these two subspecies was found in a world-

2692   wide review of variation in wolf mtDNA sequences (Vilà et al. 1999), but samples were

2693   similarly too small and widespread to be very informative about subspecies differences.

2694   The microsatellite DNA study of Carmichael et al. (2008) from widespread localities in

2695   Canada lends support for distinguishing *C. l. occidentalis* from *C. l. nubilus*, with most sampling

2696   areas largely attributable to *C. l. occidentalis* (Qamanirjuaq, Saskatchewan, Bluenose West, Cape

2697   Bathurst, Manitoba, Alberta, Porcupine, Alaska, Yukon, British Columbia, and Mackenzie)

2698   occurring together on the neighbor-joining tree (Carmichael et al 2008, Figure 3(B)). These

2699   sampling areas were not designed to assess subspecies classification, so some straddle Nowak's

2700   (1995) boundaries between the two subspecies. The Bathurst and Qamanirjuaq sampling areas

2701   appear to also include individuals from Nowak's (1995) range for *C. l. nubilus*, and this may

2702   explain why these localities do not group closely with other *C. l. occidentalis*.

2703   The SNP study of vonHoldt et al. (2011) includes samples from coastal areas of British

2704   Columbia, which is within the range of *C. l. nubilus* as mapped by Nowak (1995). It also

2705   includes samples within the general range of *C. l. occidentalis*: "boreal forest" (Alaska),

2706   "tundra/taiga" (inland Canada), and "Rocky Mountain" (Yellowstone). The western coastal

2707   sample is differentiated from other western gray wolves on some axes of the principal

2708   components analysis (vonHoldt et al. 2011, Supplementary Figure S3). In a neighbor-joining

2709   tree and a phylogram (vonHoldt et al. 2011, Supplementary Figure S5), western coastal and

2710   northern Quebec follow Mexican wolf in representing branches basal to other western gray

2711   wolves. F_{ST} among samples representing *C. l. occidentalis* (Alaska, inland Canada, and

2712   Yellowstone) range 0.01 to 0.03, while F_{ST} between these samples and the *C. l. nubilus* sample

2713   from coastal British Columbia is greater (range 0.6 to 0.8). vonHoldt et al. (2011) attribute this

2714   difference between coastal and inland populations and other patterns of geographic variation

2715   within gray wolves to differences in habitat characteristics. Differences in habitat can, however,

2716   coincide with subspecies boundaries and can play a role maintaining taxonomic distinctions

2717   when ranges of subspecies are contiguous. Explanations based on habitat variation can therefore

2718   be consistent with subspecies ranges and boundaries

2719   Wolves in the western range of *C. l. occidentalis* are genetically discontinuous with

2720   coastal wolves of British Columbia and southeast Alaska at autosomal microsatellite DNA loci

2721   and for mtDNA haplotypes (Weckworth et al. 2005, 2010; Muñoz-Fuentes et al. 2009). The

2722   coastal wolves in this area were considered to be *C. l. nubilus* by Nowak (1995, 2002) these

2723   genetic data are discussed in more detail in the following section on that subspecies.

2724   The overview of mtDNA variation presented in Table 4 indicates that *C. l. occidentalis*

2725   (Interior: Alaska, Yukon, Northwest Territories, Alberta, and Saskatchewan) and historical *C. l.*

2726   *nubilus* share some haplotypes but most, especially for *C. l. occidentalis*, are not shared.

2727   *Canis lupus mackenzii* is treated as a synonym of *C. l. occidentalis* by Nowak (1995). Its

2728   distribution is mapped by Goldman (1944) and Hall (1981) as the northern Northwest Territories

2729   extending to the Arctic Ocean. The genetic discontinuity at the Mackenzie River indicated by

2730   microsatellite DNA near the southwest boundary of the mapped range of *C. l. mackenzii*

2731   (Carmichael et al. 2001) raises the possibility of genetic support for recognizing it as a

2732   subspecies. Additional microsatellite DNA data covering the eastern portion of the range of *C. l.*

2733   *mackenzii* (Carmichael et al. 2008), which includes the type locality (Bathhurst Inlet), does not

2734   support subspecies standing because the sampling areas (Cape Bathurst, Bluenose West, and

2735   Bathurst) that represent the range of *C. l. mackenzii* do not group together in a neighbor-joining

2736   analysis (Carmichael et al. 2008, Figure 3(B)).

2737   The autosomal microsatellite DNA (Carmichael et al. 2007, 2008) and mtDNA data

2738   (summarized in Table 4) indicate limited genetic continuity between *C. l. nubilus* and *C. l.*

2739   *occidentalis*. Although there are the exceptions noted above related to sampling areas not being

2740   confined to single subspecies, these data are in general agreement with the morphometric support

2741   (Skeel and Carbyn 1977, Nowak 1995) for recognizing *C. l. occidentalis*.

2742

2743   *Canis lupus nubilus (plains wolf).* This is the most difficult and complex subspecies to evaluate

2744   because it is or was historically in contact with each of the other three *C. lupus* subspecies, *C.*

2745   *lycaon*, and probably *C. rufus*. Some areas included within *C. l. nubilus* range may represent

2746   intergrade or contact zones between subspecies. More localized genetic structure generated by

2747   habitat and prey preferences (Carmichael et al. 2001, 2007, 2008; Pilot et al. 2006; Musiani et al.

2748   2007) may alternatively obscure or coincide with older patterns of structure that may be of more

2749   taxonomic significance. Moreover, this subspecies has suffered extirpation over a great part of

2750   its range, including all of the conterminous United States except for northeastern Minnesota and

2751   Isle Royale, so genetic data have been limited by the relatively few individuals from museum

2752   collections that have been characterized.

2753   The kind of unique and divergent mtDNA haplotype sequences that have been so

2754   important in our understanding of *C. lycaon* and *C. l. baileyi* are not as evident in *C. l. nubilus*.

2755   The previously noted limitations of the size and scale of sampling of mtDNA for evaluating

2756   subspecies taxonomy are particularly applicable for evaluating *C. l. nubilus*. The most widely-

2757   distributed haplotypes, designated lu-28 and lu-32 by Vilà et al. (1999) are widely distributed in

2758   both *C. l. nubilus* and *C. l. occidentalis*. Haplotypes from museum specimens indicate that both

2759   haplotypes were widely distributed in the western United States prior to extirpation of wolves

2760   from those areas (Leonard et al. 2005). Interestingly, lu-32 and a similar haplotype were also

2761   found in historical samples from Labrador (Leonard et al. 2005), which supports the similarity of

2762   wolves within the wide geographic range attributed by Nowak (1995) to *C. l. nubilus*. It does

2763   not, however, appear to be a genetic marker unique to *C. l. nubilus*, as it occurs within the range

2764   of *C. l. occidentalis* in Alberta and Northwest Territories. Most haplotypes from historical *C. l.*

2765   *nubilus* have not been found in *C. l. occidentalis* (Table 4, where *C. l. occidentalis* is represented

2766   by "Interior" samples from Alaska, Yukon, Northwest Territories, British Columbia,

2767   Saskatchewan, and most of Manitoba).

2768   The neighbor-joining analysis of microsatellite DNA data (Carmichael et al. 2008, Figure

2769   3(A)) groups together some northern sampling areas for *C. l. nubilus*, which include Baffin

2770   Island, the adjacent mainland, and Atlantic (Newfoundland). The Bathurst sampling area is also

2771   included in this group, but as discussed under *C. l. occidentalis*, it straddles Nowak's (1995)

2772   boundary dividing the two subspecies. Unfortunately for taxonomic purposes, this study was

2773   designed to explore relationships of Arctic wolves and did not include samples from Ontario or

2774   Quebec in the southern Canada range of *C. l. nubilus*.

2775   Nowak (1995) included wolves from the Pacific Northwest of the United States, coastal

2776   British Columbia, and southeastern Alaska in *C. l. nubilus*. An affinity between wolves in

2777   western coastal areas of Canada and those in southeastern Alaska was shown by morphometric

2778   data (Jolicoeur 1959). These coastal populations are closer in morphology to one another than

2779   nearby inland populations (Jolicoeur 1959, Nowak 1983). Nowak (1995) observed that the

2780   southeastern Alaska populations are intermediate between *C. l. nubilus* and *C. l. occidentalis*.

2781   Differentiation between coastal and inland wolves in southeastern Alaska has been

2782   reported for both microsatellite DNA (Weckworth et al. 2005) and mtDNA data (Weckworth et

2783   al 2010). Differentiation between coastal and inland wolves in British Columbia has also been

2784   documented using mtDNA (Muñoz-Fuentes et al. 2009). These coastal-inland patterns of

2785   divergence lend support to Nowak's (1995) boundary between *C. l. nubilus* and *C. l. occidentalis*

2786   in this region. The neighbor-joining tree of Carmichael et al. (2008) places southeast Alaska

2787   wolves with populations attributable to *C. l. occidentalis* rather than the distant, eastern *C. l.*

2788   *nubilus* represented in the study. Microsatellite DNA data may not be reliable, however, in

2789   situations where there is this type of geographic discontinuity among populations (Paetkau et al.

2790   1997).

2791   Coastal populations of southeast Alaska (Weckworth et al. 2010) and British Columbia

2792   (Muñoz-Fuentes et al. 2009) form a distinct phylogroup based on mtDNA, and share the

2793   common lu38 and unique lu68 haplotypes (Table 4). This sharing of haplotypes argues against

2794   taxonomically distinguishing the southeast Alaska wolves (as *C. l. ligoni*) from the coastal

2795   populations of British Columbia.

2796   Although Nowak (1995) assigned the coastal populations of southeast Alaska and British

2797   Columbia to *C. l. nubilus*, genetic comparison with inland *C. l. nubilus* populations of the

2798   western United States is limited to the relatively small sample of historical individuals for which

2799   genetic data (mtDNA) are available (Leonard et al. 2005). The finding of Muñoz-Fuentes (2009)

2800   that coastal British Columbia wolves are less differentiated from inland *C. l. occidentalis* ($\Phi_{ST} =$

2801   0.305) than from the historical samples (Leonard et al. 2005) of *C. l. nubilus* from the

2802   conterminous western United States (Φ_{ST} = 0.550) led them to assert that this analysis supported

2803   their view that these coastal wolves were evolutionarily distinct from inland wolves, including *C.*

2804   *l. nubilus*. However, the large proportion of unique, and apparently extinct, haplotypes in the

2805   historical sample contributes to an exaggerated measure of divergence between the coastal

2806   populations and historical inland *C. l. nubilus*. A different picture emerges when examining the

2807   phylogenetic relationships of the haplotypes. The most common haplotype (lu38) in coastal

2808   British Columbia also occurs in historical Kansas and Nebraska samples (Table 4, Leonard et al.

2809   2005), and nearly all coastal haplotypes are in the same phylogroup as the historical western *C. l.*

2810   *nubilus* haplotypes (Weckworth et al. 2010, Figure 2). These relationships are consistent with

2811   the coastal British Columbia and southeast Alaska wolves being a northward extension of *C. l.*

2812   *nubilus.*

2813   The wolves of southeast Alaska are the descendents of a southern Pleistocene refugium

2814   or southern clade (*C. l. nubilus*) that migrated north along the west coast as glacial ice retreated

2815   inland some 12,000 years ago. The northern continental wolves of the inland localities in this

2816   study are an admixture of northern refugium wolves from Beringia (*C. l. occidentalis*) with a

2817   substantial contribution from southern clade wolves that migrated north as glacial ice retreated

2818   from the Great Plains. Northwest coastal wolves are a vestige of a widespread southern

2819   phylogroup that was extirpated during the last century.

2820   The Pacific Coastal region of British Columbia and southeastern Alaska is occupied by

2821   very closely-related populations of wolves that share phylogenetic relationships and certain

2822   haplotypes with the historical *C. l. nubilus* of inland portions of the western United States. The

2823   name *Canis lupus crassodon* (type locality: Tahsis Canal, Vancouver Island) has been used for

2824   the wolves of Vancouver Island (Hall 1981), but mtDNA data do not indicate differentiation

2825 between modern samples from Vancouver Island and the coastal mainland of British Columbia

2826 (Muñoz-Fuentes et al. 2009), and the extirpation of the original population followed by

2827 colonization by wolves of a different mtDNA haplotype (Muñoz-Fuentes et al. 2010) further

2828 argues against taxonomic distinction of the modern wolf population of the island. Furthermore,

2829 the haplotype lu68 found in historical wolves from Vancouver Island is common in coastal

2830 mainland wolves today, so there is no genetic support for taxonomic distinction of the historical

2831 population of the island.

2832   The wolf population of coastal British Columbia was probably contiguous with the

2833 original populations of coastal Washington and Oregon, which were included by Goldman

2834 (1944) with *Canis lupus fuscus*, the type locality of which (near The Dalles, Oregon) was not

2835 coastal. Hall and Kelson (1959) included most of coastal British Columbia with the range of this

2836 subspecies, which Nowak considered a synonym of *C. l. nubilus*. Bailey (1936) identified

2837 coastal wolves of Oregon as *Canis lycaon gigas* (type locality near Vancouver, Washington).

2838 Goldman (1944) included this name as a synonym of *Canis lupus fuscus*. Genetic study of

2839 historical individuals from western Oregon and Washington would be valuable in evaluating the

2840 alternative taxonomic placements based on traditional morphology and morphometrics of the

2841 historical population of the Pacific Northwest of the United States.

2842   Another portion of the range whose subspecies assignment is uncertain is the area west of

2843 Hudson Bay in Northwest Territories. Skeel and Carbyn (1977) found morphometric affinity

2844 with *C. l. occidentalis* from Wood Buffalo National Park, the basis of which Nowak (1995) has

2845 questioned on the basis of their inclusion of females with samples of males. The Qamanirjuaq

2846 sampling area of the microsatellite DNA study of Carmichael et al. (2008) encompasses an area

2847 from Hudson Bay west, and it straddles the boundary between the two subspecies. As a possible

2848 consequence, its position on the neighbor-joining tree (Carmichael et al. 2008, Fig 3(B)) is only

2849 weakly supported and therefore provides little reliable information on taxonomic standing.

2850   Hybrid or intergrade zones involving *C. l. nubilus* were discussed in sections on

2851 relationships of *C. lycaon* to gray wolves and *C. l. baileyi*. General conclusions on these

2852 intergrade zones are repeated here. *Canis lupus nubilus* forms a hybrid zone with *C. lycaon* from

2853 eastern Ontario to Minnesota and Manitoba. The hybrid zone includes wolves that have recently

2854 re-colonized Wisconsin and the Upper Peninsula of Michigan. There was historical contact

2855 between *C. l. nubilus* and *C. l. baileyi*. Information from historical specimens indicates that

2856 haplotypes of *C. l. nubilus* occurred as far south as Arizona and *C. l. baileyi* haplotypes occurred

2857 as far north as Nebraska. Wolves were subsequently extirpated from the area of this zone, and

2858 the only wolves now present near this area are *C. l. baileyi* that have been reintroduced in

2859 southern Arizona and New Mexico.

2860   Nowak's (1983, 1995) classification and evolutionary explanation characterizes *C. l.*

2861 *nubilus* as a medium-size wolf that was widespread in North America at the time of arrival of the

2862 larger *C. l. occidentalis*. Morphometric analyses by Skeel and Carbyn (1977) provide general

2863 support for a comparable distribution of larger and smaller wolves in central Canada. Autosomal

2864 microsatellite DNA data (Carmichael et al. 2007, 2008) distinguish *Canis lupus nubilus* from *C.*

2865 *l. occidentalis* in the northeastern portion of its range, and both microsatellite and mtDNA data

2866 (Weckworth et al. 2005, 2010; Muñoz-Fuentes et al. 2009, 2010) distinguish its Pacific Coast

2867 populations from inland *C. l. occidentalis*. Historical samples of *C. l. nubilus* from the western

2868 United States (Leonard et al. 2005, Table 4 of this paper) have several unique and

2869 phylogenetically related mtDNA haplotypes. The available genetic information bearing on the

2870   question of subspecies lends general support for *C. l. nubilus* as delineated by Nowak (1995,

2871   2003), at least in the areas covered by those studies.

2872

2873   **A General Evolutionary Interpretation**

2874   The following evolutionary scenario is presented as an overview of the conclusions of

2875   this review in the context of the evolutionary history of modern North American *Canis*.

2876   Coyotes, *Canis rufus*, and *Canis lycaon* are modern representatives of a major and diverse clade

2877   that evolved within North America, as proposed by Wilson et al. (2000). *Canis lupus* arose in

2878   Eurasia and invaded North America at least three separate times, each invasion by one or more

2879   different clades of Eurasian *Canis lupus*. The first of these invasions was by the ancestors of *C.*

2880   *l. baileyi*, as suggested by Vilà et al. 1999, followed by the invasion by the ancestors of *C. l.*

2881   *nubilus*, which displaced *C. l. bailey* in the northern part of its range. While expanding in North

2882   America and displacing *C. l. bailey*, the historical *C. l. nubilus* population gained some mtDNA

2883   haplotypes from the latter (Leonard et al. 2005) in a process whereby an invading population is

2884   genetically introgressed with local genes. The distribution of *C. l. occidentalis* has the general

2885   form of an invading population, and its southward expansion and displacement of *C. l. nubilus*

2886   may have continued into historic times. The final invasion, probably post-glacial, was by *C. l.*

2887   *occidentalis*, which displaced *C. l. nubilus* in the northern part of its former range. This final

2888   phase was undoubted more complex, as the biogeography of Beringia is complex, and at least

2889   one Beringian lineage of *C. lupus* became extinct without leaving genetic traces in modern

2890   wolves (Leonard et al. 2007). *Canis lupus* is not morphologically or genetically homogeneous or

2891   undifferentiated across North America. An interpretation that wolves of these different lineages

2892   have mixed in North America to an extent that the only geographic pattern is isolation by

2893   distance is not supported by the geographic distribution of lineage markers. There is geographic

2894   structure in genetic composition (Tables 4, 5) that is consistent with multiple invasions of North

2895   America from Eurasia. This geographic structure on a continental scale coincides with the

2896   general distributions of the three *C. lupus* subspecies recognized in this review.

2897

2898   **Final Comments and Recommendations**

2899   The taxonomic recommendations and conclusions stated here are intended to represent

2900   the most reasonable interpretations based on the available scientific information. A very

2901   generalized summary of the findings are provided on a map in Figure 13 of this paper. Some

2902   conclusions, such as the taxonomic standing of *Canis lupus baileyi*, are more strongly supported

2903   than others. The taxonomic standing for *Canis lupus arctos* is not confirmed, but important

2904   limitations in the available data do not permit more definitive statements on its taxonomic status.

2905   It is possible that further research will provide data that would change certain conclusions

2906   reached here. Longer sequences of mtDNA (most studies used ~200 to ~400 base pairs) could

2907   provide more robust resolution of both extant and historical populations. There are many more

2908   specimens from museum and government agency collections that have not yet had DNA

2909   characterized. Y-chromosome haplotypes from additional populations of wolves would provide

2910   an additional lineage marker to complement mtDNA data. Single nucleotide polymorphisms

2911   (SNPs) are now being studied in wolves, but only a small amount of preliminary data on wild

2912   populations have been reported (Anderson et al. 2009, Gray et al. 2009). Genomic approaches

2913   have potential to provide vast amounts of information on individual specimens, but it is not yet

2914   known how they will affect our understanding of relationships among populations and their

2915   taxonomic standing.

2916   Even with expanded application of various genetic markers, geographic coverage or

2917   spatial sampling patterns may still limit our understanding of crucial areas. There are sampling

2918   concerns on both very broad and more localized scales. A notable example on the broadest scale

2919   is the original wolf population of the conterminous United States, which was extirpated outside

2920   of northeastern Minnesota and Isle Royale. Without genetic study of additional specimens from

2921   collections or possibly as yet unstudied or undiscovered natural deposits of bones or other

2922   persistent remains of wolves, the coverage of crucial and underrepresented geographic areas will

2923   hinder our understanding of the historical relationships of populations. Additional morphometric

2924   studies that use more objective methods and explore more alternative taxonomic arrangements

2925   have potential for improving our understanding of evolutionary relationships and their taxonomic

2926   implications, especially when integrated with genetic data.

2927   The design of spatial sampling is also important on a more local scale (Schwartz and

2928   McKelvey 2009, Schwartz and Vucetich 2009). This is particularly important in cases where

2929   both isolation by distance and local barriers (ecological or behavioral) to interaction are

2930   suspected to be operating, as in the Great Lakes region. Grouping of spatial genetic data by state

2931   or province, for example, can obscure finer-scale patterns of contact and interaction between

2932   populations with different evolutionary histories. Geographic mapping of haplotypes in the

2933   Great Lakes region (Fain et al. 2010, Wheeldon et al. 2010) provides a more objective portrayal

2934   of these interactions and facilitates the identification of areas for further investigation.

2935   There is scientific support for the taxa recognized here, but delineation of exact

2936   geographic boundaries presents challenges. Rather than sharp lines separating taxa, boundaries

2937   should generally be thought of as intergrade zones of variable width. These "fuzzy" boundaries

2938   are a consequence of lineages of wolves that evolved elsewhere coming into contact. Historical

2939   or modern boundaries should also not be viewed as static or frozen in any particular time. Our

2940   understanding of the historical interactions between subspecies or genetically different

2941   populations (e.g., Leonard et al. 2005) is that they are dynamic processes and boundaries can

2942   shift over time. Even with the great dispersal capabilities of wolves and their interaction in these

2943   intergrade zones, genetic indications of the independent evolution of the wolves here recognized

2944   as species or subspecies are still discernable on a continental scale.

2945   As stated in the Scope and Intent section at the beginning of this review, we have not

2946   evaluated non-taxonomic alternatives to subspecies classifications of wolves, such as

2947   management units or evolutionarily significant units, or the appropriateness of legal protection as

2948   distinct vertebrate population segments under the Endangered Species Act (USFWS and NOAA

2949   1995). Even with scientific support of its taxonomic validity, a subspecies may or may not be

2950   the most suitable unit for protection or management. Weighing the value of subspecies

2951   compared to non-taxonomic units requires the evaluation of specific legal, policy, and

2952   management objectives that are decidedly beyond the scope of this review. We are aware that

2953   taxonomy can have policy and legal implications, but we have carried out his review following

2954   the rule that, "[t]he relationship between conservation and taxonomy must be unidirectional;

2955   conservation strategies should be influenced by taxonomy, but taxonomy cannot be influenced

2956   by conservation priorities" (Bowen and Carl 1999). This review was prepared to provide

2957   objective evaluations of controversial issues in wolf taxonomy for the consideration of those who

2958   have the responsibility for using the best available scientific information in concert with legal

2959   and policy considerations in developing conservation programs.

2960   This review was initiated because of the wide range of views expressed by different

2961   researchers and research groups on some major features of relationships and classification of

2962　North American wolves. We have endeavored to be as comprehensive and objective as possible

2963　in developing recommendations based on the total information available today, and have sought

2964　to reconcile differing interpretations in the literature whenever possible.

2965

2979

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3369   Table captions:

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3371   Table 1. Available names for North American "gray" wolves in order of their publication date

3372   (does not include coyotes). Based on Goldman (1944), Hall and Kelson (1959) and Hall (1981).

3373

3374   Table 2. Subspecies of *Canis lupus* recognized by Hall and Kelson (1959) and Hall (1981),

3375   largely based on Goldman (1944). The five subspecies recognized by Nowak (1995) in his

3376   revision are in **bold** and each is followed by its synonyms as recognized in his reclassification.

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3378   Table 3. Subspecies of *Canis rufus* (Goldman 1944, Nowak 1979, Hall 1981).

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3380   Table 4. Number of individuals identified for 35 mtDNA-control region sequence haplotypes

3381   found in wolves from coastal locations in Alaska (AK) and British Columbia (BC) and interior

3382 locations in Alaska, Yukon, Northwest Territories (NWT), British Columbia, Alberta (ALTA),

3383 Saskatchewan (SASK), Manitoba (MAN), Minnesota, Wisconsin and Michigan (GLS) and

3384 eastern Ontario (EONT). The historical *C. l. nubilus* samples were combined from locations in

3385 North Dakota, Colorado, Kansas, Nebraska, Oklahoma and New Mexico; and the historical *C. l.*

3386 *baileyi* samples were combined from locations in Arizona, New Mexico and northern Mexico

3387 (Leonard et al 2005).

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3389 Table 5. Number of individuals identified for 33 Y-chromosome haplotypes found in wolves

3390 from locations in Alaska (AK), Northwest Territories (NWT), British Columbia (BC), Alberta

3391 (ALTA), Great Lakes states of Minnesota, Wisconsin and Michigan (GLS), Texas (TX) as well

3392 as dog breeds originating in the Americas (NW) and Europe (OW). Haplotypes were derived

3393 from the dog Y-chromosome loci MS34A, MS34B, MS41A and MS41B (Olivier & Lust 1998,

3394 Sundqvist et al 2001).

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3396 Table 6. Genetic distances based on microsatellite DNA between paired samples of eastern wolf

3397 (Southern Quebec, Algonquin, western Great Lakes), other wolves, and coyotes. Genetic

3398 distances between samples with substantial eastern wolf representation are indicated in bold.

3399 Koblmüller et al. (2009a) used the notation Θ_{ST} to report the coancestry parameter Θ of Weir and

3400 Cockerham (1984), which they consider to be generally comparable to F_{ST}, the fixation index of

3401 Wright (1951). *D* is the unbiased genetic distance of Nei (1978).

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3403 Table 7. Private alleles among wolf populations with respect to populations covered in each

3404 cited source. Values representing eastern wolf are in bold.

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3406   Table 8. Mitochondrial DNA sequence divergences.

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3408   Table 9. Mitochondrial DNA sequence divergences within the coyote clade

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3428 Table 1.

Species or subspecies	Author(s)	Date	Described as:	
			Species	Subspecies
Canis lycaon	Schreber	1775	X	
Canis nubilus	Say	1823	X	
Canis lupus occidentalis	Richardson	1829		X
Canis lupus fuscus	Richardson	1839		X
Canis lupus canadensis	Blainville	1843		X
Lupus gigas	Townsend	1850	X	
Canis lupus var. *rufus*	Audubon & Bachman	1851		X
Canis occidentalis griseoalbus	Baird	1858		X
Canis pambasileus	Elliot	1905	X	
Canis floridanus	Miller	1912	X	
Canis tundrarum	Miller	1912	X	
Canis nubilus baileyi	Nelson & Goldman	1929		X
Canis occidentalis crassodon	Hall	1932		X
Canis lupus arctos	Pocock	1935		X
Canis lupus orion	Pocock	1935		X
Canis rufus gregoryi	Goldman	1937		X
Canis lupus beothucus	Allen & Barbour	1937		X
Canis lupus labradorius	Goldman	1937		X
Canis lupus ligoni	Goldman	1937		X
Canis lupus youngi	Goldman	1937		X
Canis lupus irremotus	Goldman	1937		X
Canis lupus monstrabilis	Goldman	1937		X
Canis lupus mogollonensis	Goldman	1937		X
Canis tundrarum ungavensis	Comeau	1940		X
Canis lupus alces	Goldman	1941		X
Canis lupus columbianus	Goldman	1941		X
Canis lupus hudsonicus	Goldman	1941		X
Canis lupus bernardi	Anderson	1943		X
Canis lupus mackenzii	Anderson	1943		X
Canis lupus manningi	Anderson	1943		X
Canis lupus knightii	Anderson	1947		X

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3432   Table 2.

Subspecies	Author(s)	Date	Type locality
Canis lupus lycaon	Schreber	1775	Restricted by Goldman (1937) to vicinity of Quebec, Quebec
Canis lupus baileyi	Nelson & Goldman	1929	Colonia Garcia (about 60 miles SW of Casas Grandes), Chihuahua, Mexico (altitude 6,700 feet)
Canis lupus nubilus	Say	1823	Near Blair, Washington County, Nebraska
Canis lupus fuscus	Richardson	1839	Banks of Columbia river below The Dalles, Oregon
Canis lupus crassodon	Hall	1932	Tahsis Canal, Nootka Sound, Vancouver Island, British Columbia
Canis lupus beothucus	Allen & Barbour	1937	Newfoundland
Canis lupus labradorius	Goldman	1937	Fort Chimo, Quebec
Canis lupus ligoni	Goldman	1937	Head of Duncan Canal, Kupreanof Island, Alexander Archipelago, Alaska
Canis lupus youngi	Goldman	1937	Harts Draw, N. slope of Blue Mountains, 20 miles NE Monticello, San Juan County, Utah
Canis lupus irremotus	Goldman	1937	Red Lodge, Carbon County, Montana
Canis lupus monstrabilis[1]	Goldman	1937	Ten miles S of Rankin, Upton County, Texas
Canis lupus mogollonensis[1]	Goldman	1937	S. A. Creek, 10 miles NW Luna, Catron County, New Mexico
Canis lupus hudsonicus	Goldman	1941	Head of Schultz Lake, Keewatin, [now Nunavut], Canada
Canis lupus manningi	Anderson	1943	Hantzsch River, E side Foxe Basin, W side Baffin Island, District of Franklin, Northwest Territories [now Nunavut], Canada
Canis lupus arctos	Pocock	1935	Melville Island, Canadian Arctic
Canis lupus orion	Pocock	1935	Cape York, northwestern Greenland
Canis lupus bernardi	Anderson	1943	Cape Kellett, Banks Island, Northwest Territories, Canada
Canis lupus occidentalis	Richardson	1829	Restricted by Miller (1912) to Fort Simpson, Mackenzie, Canada
Canis lupus griseoalbus	Baird	1858	Restricted by Hall and Kelson (1952) to Cumberland House, Saskatchewan

Canis lupus pambasileus	Elliot	1905	Upper waters of Sushitna River, Region of Mount McKinley
Canis lupus tundrarum	Miller	1912	Point Barrow, Alaska
Canis lupus alces	Goldman	1941	Kachemak Bay, Kenai Peninsula, Alaska
Canis lupus columbianus	Goldman	1941	Wistaria, N side of Ootsa Lake, Coastal District, British Columbia.
Canis lupus mackenzii	Anderson	1943	Imnanuit, W of Kater Point, Bathurst Inlet, District of Mackenzie, Northwest Territories

[1]Considered synonyms of *Canis lupus baileyi* Goldman, 1937 by Bogan and Mehlhop (1983)

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3450   Table 3.

Subspecies	Author(s)	Date	General range (Nowak, 2002)
Canis rufus rufus	Audubon & Bachman	1851	Central and Gulf coast Texas & Louisiana
Canis rufus floridanus	Miller	1912	United States east of the Mississippi River
Canis rufus gregoryi	Goldman	1937	Northeast Texas to Indiana

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3470 Table 4.

Haplotype	Genbank	Coastal (n=277)			Interior (n=966)									Historical samples (n=84)		
		AK	SEAK	BC	AK	Yukon	NWT	BC	ALTA	SASK	MAN	GLS	EONT	C. l. nubilus	C. l. baileyi	C. lycaon
lu38[a,h], F[b], I[b]	AF812731		127[b]	57[a]	3[a]		4[a]	23[a],5[b]						3[h]		
lu48[h]	AY812733													1[h]		
lu49[h]	AY812734													1[h]		
lu68[a,l], H[b]	FN298179		2[b]	14[a]										12[l]		
lu32[a,h], W6[c], C22[d,i]	AF005309			4[a]	1[a]		25[a],232[g]	31[a]	20[c],1[f]	4[d]	10[d], 1[i]	38[c]	4[e]	3[h]	2[h]	
lu53[h]	AY812738													1[h]		
lu54[h]	AY812739													2[h]		
lu11[a]	AF005300						2[a]									
lu28[a,h], A[b], M[b], W7[c], C	AF005308	54[b]			12[a],41[b],?	3[b]	1[a]	15[a],7[b]			25[d]	1[c]	3[i]	7[h]		
lu52[h]	AY812737													1[h]		
lu67[a]	FM201672							1[a]								
lu29[a,f], B[b]	AF005310	18[b]			5[a],8[b]	6[b],1[f]	3[a],57[g]	2[a]								
lu30[a,f], L[b]	AF005311				4[a]	2[b],1[f]		1[b]								
lu31[a,f], K[b], W1[c]	AF005312				4[a],3[b]	1[f]	4[a]	11[a],10[b]	5[c]							
lu37[a]	AF812730				2[a],3[b]											
lu61[a], C[b], J[b], 16[d], W3[c]	AF812741	1[b]			3[a],9[b]		1[a]				20[d]	1[d]				
lu36[a]	FM201632							2[a]								
G[b]	GQ376506					1[b]										
N[b]	GQ376226							1[b]								
lu33[h]	AF005313														4[h]	
lu47[h]	AY812732														1[h]	
lu50[h]	AY812735														6[h]	
lu51[h]	AY812736														1[h]	
C3[d,e], C21[c], GL2[k,m]	FJ213916									4[d▲]	18[e▲]					5[k],2[m]
C13[e,j,n], C12[c], GL10[m]	FJ213915										58[e▲]		7[e▲]			1[j],1[m],1[n]
C1[e,n], C4[e], GL1[k,m]	FJ213914											1[c▲]	28[e▲], 7[i]			7[i],1[j],5[k],1[m],1[n]
C9[e]	AY267726												62[eΔ]			1[i]
C14[e]	AY267731												52[eΔ]			3[i]
C16[e]	AY267733												1[eΔ]			
C17[e]	AY267734												10[eΔ]			1[i]
C19[e]	AY267736												38[eΔ]			1[i]
GL3[k]	J. Leonard[PC]															1[k]
GL5[k,m]	J. Leonard[PC]															1[k],1[m]
GL6[k,m]	J. Leonard[PC]															1[k],1[m]
GL8[k,m]	J. Leonard[PC]															1[k],1[m]
Total		73[b]	129[b]	75[a]	34[a],64[b],3[f]	12[b],3[f]	40[a],289[g]	85[a],24[b]	25[c],1[f]	24[d]	1[i],36[d],4[d▲]	39[c],77[c▲]	3[i],4[e],35[e▲],163[eΔ]	19[h],12[l]	14[h]	13[i],2[j],14[k],7[m],3[n]

[a]Muños-Fuentes et al (2009a), [b]Weckworth et al (2010), [c]Fain et al (2010), [d]Stronen et al (2010), [e]Grewel et al (2004), [f]Vilá et al (1999), [g]Musiani et al (2007), [h]Leonard et al (2005), [k]Leonard and Wayne (2008), [l]Muñoz-Fuentes et al (2009b), [m]Koblmüller et al (2009), [n]Wheeldon and White (2009)

▲*C. lycaon* haplotype ([i]Wilson et al 2000, [j]Wilson et al 2003), Δ*C. latrans* haplotype (Wilson et al 2000), [PC]Sequence not available on GenBank - obtained by personal communication with J. Leonard

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3480   Table 5.

Haplotype	Locality										
	AK C. lupus	NWT C. lupus	BC C. lupus	ALTA C. lupus	GLS C. lupus	GLS C. lycaon	C. l. baileyi [f]	C. rufus [f]	C. latrans TX	C. familiaris NW, OW	Totals
E[e]						29[e]					29
O[e]						2[e]					2
R[e]						10[e]					10
Y[e]						1[e]					1
X[e]						1[e]					1
H7[a], H1[d]								1[d]		202[a]	203
H15[d]								4[d]	2[d]		6
H28[d], G[e]	3[e]						6[d]				9
H29[d]							10[d]			110[a]	120
H30[bc], I[e]	1[b], 1[e]	32[c]	5[e]	12[e]							51
H31[bc]		1[b], 8[c]									9
H32[bc], Z[e]	3[b],	2[b], 21[c],			7[e]						33
H33[bc], A[e], U	3[b], 1[e]	18[c]	A1[e], U2[e]		A6[e], U10[e]						41
H34[bc]		6[b], 19[c]									25
H35[bc], W[e]		2[b], 32[c]			1[e]						35
H36[bc], F[e]	2[b], 1[e]	20[c]		1[e]	4[e]						28
H37[b]	2[b]										2
H38[bc], J[e]		1[b], 28[c]	3[e]	2[e]	4[e]						38
H39[bc], L[e]	1[b], 1[e]	1[b],									3
H40[b]		1[b]									1
H41[c]		2[c]									2
H44[c]		1[c]									1
H45[c]		1[c]									1
H50[c]		17[c]									17
H52[c]		5[c]									5
H53[c]		1[c]									1
H55[c], D[e]		1[c]			2[e]						3
H58[c]		2[c]									2
H59[c]		1[c]									1
B[c]			1[c]								1
Ff[e]			2[e]								2
M[e]	1[e]										1
Total	20	223	14	15	34	43	16	5	2	312	684

[a]Bannasch et al (2005), [b]Sundqvist et al (2006), [c]Musiani et al (2007), [d]Hailer & Leonard (2008), [e]Fain et al (2010), [f]Experimental population

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3485   Table 6.

Comparison	Distance	Measure	Source
Southern Quebec - Minnesota	**0.135**	D	Roy et al. 1994
Southern Quebec - northern Quebec	0.296	"	"
Southern Quebec - western *C. lupus*	0.251 to 0.519	"	"
Great Lakes modern - Great Lakes historic	**0.057**	Θ	Koblmüller et al. 2009a
Great Lakes modern - western wolves	0.075	"	"
Great Lakes modern - eastern coyotes	0.142	"	"
Great Lakes modern - western coyotes	0.133	"	"
Algonquin - proximal wolf populations	**0.024 to 0.055**	F_{ST}	Grewal et al. 2004
Algonquin - Abitibi Temiscamingue	0.089	"	"
Algonquin - La Verendrye Reserve	0.091	"	"
Algonquin - northeast Ontario	0.076	"	"
Algonquin - western Great Lakes states	**0.164**	"	"
W. Great Lakes States - NE Ontario & Quebec	**0.068**	"	Wheeldon 2009
Algonquin - Frontenac Axis	0.109	"	"
Algonquin - NE Ontario & Quebec	0.135	"	"
Algonquin - Manitoba	0.232	"	"
Algonquin - Northwest Territories	0.238	"	"
Algonquin - Southern Magnetawan	**0.022**	"	Wilson et al. 2009
Algonquin - Frontenac Axis	0.055	"	"
Algonquin - northwest Ontario	0.071	"	"
Algonquin - northeast Ontario	0.073	"	"
Algonquin - Minnesota	**0.089**	"	"
Algonquin - Pukaskwa National Park	0.117	"	"
Among Minnesota, Wisconsin, and Michigan	**0.006 to 0.016**	"	Fain et al. 2010
Western Great Lakes states - western wolves	0.125	"	"
Western Great Lakes states - Wisconsin coyotes	0.159	"	"
Algonquin - northeast Ontario	0.105	"	Rutledge et al. 2010b
Algonquin - Frontenac Axis	0.052	"	"
Frontenac Axis - northeast Ontario	0.120	"	"

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3489   Table 7.

| | Number of | | | |
Sample	Private alleles	Loci	Individuals in sample	Source
Southern Quebec	**5**	10	24	Roy et al. 1994
Minnesota	**12**	"	20	"
Northern Quebec	1	"	20	"
Alberta	1	"	20	"
Vancouver Island	0	"	20	"
Northwest Territories	3	"	24	"
Kenai, Alaska	0	"	19	"
Western Great Lakes states	**14**	8	124	Fain et al. 2010
Alberta	2	"	26	"
British Columbia	0	"	41	"
Alaska	2	"	39	"
Algonquin	**4**	12	128	Rutlege et al. 2010b
Northeast Ontario	5	"	51	
Frontenac Axis	4	"	38	
Alaska and western Canada	5[a]	11	221	Weckworth et al. 2005
W. Montana (from Alberta founders)	6	10	91	Forbes and Boyd (1996)

[a]Average per population.

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3500   Table 8.

Comparison	No. of comparisons	% sequence divergence		Sequence source	Source
		Mean	Range or s.d.		
Gray wolf - gray wolf	1	0.6	NA	Restriction sites	Lehman et al. 1991
Coyote - coyote	3	1.5	0.8 to 2.0	"	"
Eastern wolf - eastern wolf	1	0.9	NA	"	"
Gray wolf - eastern wolf	4	3.6	3.5 to 3.6	"	"
Red wolf - red wolf	3	0.6	0.4 to 0.9	Cytochrome *b*	Roy et al. 1996
Coyote - coyote	10	1.1	0.4 to 1.7	"	"
Gray wolf - Gray wolf	3	0.9	0.4 to 1.3	"	"
Gray wolf - coyote	15	4.6	3.9 to 5.6	"	"
Gray wolf - Missouri & Oklahoma red wolf*	11	2.3	0 to 4.7	"	"
Gray wolf - Arkansas red wolf*	9	4.2	3.2-5.2	"	"
Major coyote clades	Not reported	1.7	Not reported	Control region	Wilson et al. 2000
Eastern wolf - coyote	"	3.2	"	"	"
Eastern wolf - gray wolf	"	8.0	"	"	"
Red wolf - gray wolf	"	8.0	"	"	"
Gray wolf - coyote	"	10.0	"	"	"
Historical eastern wolf clades	Not reported	Not reported	0.5 to 4.5	Control region	Leonard and Wayne 2008
Historical eastern wolf - coyotes	"	6.4	± 2.9 s.d.	"	"
Historical eastern wolf - gray wolf	"	19.1	± 5.1 s.d.	"	"
Eastern wolf - eastern wolf	Not reported	1.5	± 0.8 s.d.	Control region	Wheeldon 2009
Coyote - coyote	"	2.4	± 0.9 s.d.	"	"
Gray wolf - gray wolf	"	2.0	± 0.8 s.d.	"	"
Eastern wolf - gray wolf	"	14.8	± 6.9 s.d	"	"
Eastern wolf - coyote	"	4.7	± 1.9 s.d.	"	"
Gray wolf - coyote	"	25.4	± 11.7 s.d.	"	"
*Red wolf samples were from historical specimens					

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3506   Table 9.

Comparison:	No. of comparisons Comparisons	% sequence divergence		Sequence source	Source
		Mean	Range		
Coyote-coyote	3	1.5	0.8 to 2.0	Restriction sites	Lehman et al. 1991
Eastern wolf-coyote	6	1.5	0.9 to 2.0	"	"
Coyote-coyote	10	1.1	0.4 to 1.7	Cytochrome *b*	Roy et al. 1996
Red wolf-coyote	15	1.2	0.9 to 2.2	"	"
Coyote-coyote	Not reported	1.7	Not reported	Control region	Wilson et al. 2000
Red wolf-coyote	"	2.3	"	"	"
Eastern wolf-coyote	"	3.2	"	"	"
Red wolf-eastern wolf	"	2.1	"	"	"
Coyote-coyote	Not reported	2.8	Not reported	Control region	Adams et al. 2003
Red wolf-coyote	"	3.2	"	"	"

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3520 Figure captions:

3521 Figure 1. Ranges of gray wolves in the conterminous United States: (a) historical range; (b)

3522 range at time of listing (1978); (c) current range in the Great Lakes states and experimental

3523 population areas in the northern Rocky Mountains and for the Mexican wolf (*Canis lupus*

3524 *baileyi*) in the Southwest. Credit: U.S. Fish and Wildlife Service.

3525

3526 Figure 2. Ranges of North American *Canis lupus* subspecies recognize by Nowak (1995, 2002)

3527 and of *Canis rufus* (after Nowak 2002).

3528

3529 Figure 3. Subspecies of *Canis lupus* recognized by Goldman (Figure 14 of Goldman 1944 in

3530 *The Wolves of North America*, S. P. Young and E. A. Everman, editors, © Wildlife Management

3531 Institute; reproduced with permission).

3532

3533 Figure 4. Principal components analysis of gray wolf skulls from central Canada: projection of

3534 male groups on first three principal components (Figure 2 of Skeel and Carbyn 1977). Points

3535 labeled H, I, and I are from locations within the range of *C. l. nubilus*; L is *C. l. lycaon*; and W,

3536 R, J, and P are from within the range of *Canis lupus.* Credit: M. A. Skeel and L. N. Carbyn, The

3537 morphological relationship of Gray Wolves (*Canis lupus*) in national parks of central Canada,

3538 Canadian Journal of Zoology 55(4):737-747, © 2008 NRC Canada or its licensors. Reproduced

3539 with permission.

3540

3541   Figure 5. Principle components plot of skulls from male *Canis lupus* from the southwestern

3542   United States. (Figure 2 of Bogan and Mehlhop 1983). B, *C. l. baileyi*; M, *C. l. mogollonensis*;

3543   S, *C. l. monstrabilis*. Credit: Museum of Southwestern Biology, University of New Mexico.

3544

3545   Figure 6. Discriminant function analysis of skulls of some North American *Canis lupus* (Figure

3546   7 of Nowak 1995). Axes represent first (horizontal) and second (vertical) canonical variables.

3547   Solid lines are limits of the Nowak's southern group (corresponding to *C. l. nubilus*), which is

3548   the polygon on the left with center N; and northern group (corresponding to *C. l. occidentalis*),

3549   which is the polygon on the right with center O.   Dots represent individuals of *C. l. baileyi*.

3550   Credit: © Ronald M. Nowak and Canadian Circumpolar Institute Press. Used with permission.

3551

3552   Figure 7. Multi-dimensional scaling plot based on 10 microsatellite DNA loci (Figure 3 of Roy

3553   et al. 1996). Confidence ellipses are indicated by dashed (95%) and solid (99%) lines. © John

3554   Wiley and Sons. Used with permission.

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3556   Figure 8. Neighbor-joining tree based on mtDNA control region sequences of *Canis lupus* from

3557   Vilà et al. (1999, Figure 1). North American haplotypes are lu-28, lu-29, lu-30, lu-31, lu-32, lu-

3558   33. Others are from Eurasia. The haplotype unique to *C. l. baileyi* is lu-33. © John Wiley and

3559   Sons. Used with permission.

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3561   Figure 9. Minimum spanning tree for control region haplotypes from eastern wolves (Figure

3562   5(A) of Wilson et al. 2000). *Canis lycaon* haplotypes are C1, C3, C9, and C14; it shares C19

3563   with *C. rufus* and with coyotes. *C. rufus* has haplotypes C2 and C19. The remaining are coyote

3564   haplotypes not found in either wolf. Credit: P. J. Wilson et al., DNA profiles of eastern

3565   Canadian wolf and the red wolf provide evidence for a common evolutionary history

3566   independent of the gray wolf, Canadian Journal of Zoology 78(12):2156-2166. © 2008 NRC

3567   Canada or its licensors. Reproduced with permission.

3568

3569   Figure 10. Neighbor-joining tree of mtDNA control region haplotypes (Figure 5(B) of Wilson et

3570   al. 2000). The long branch extending to the right (C22, C23, C24) represents *Canis lupus*. *Canis*

3571   *lycaon* and *Canis rufus* haplotypes are as identified in the caption for Figure 9 of this paper.

3572   Remaining haplotypes are coyotes. Scale represents 0.1 (or 10%) sequence divergence. Credit:

3573   P. J. Wilson et al., DNA profiles of eastern Canadian wolf and the red wolf provide evidence for

3574   a common evolutionary history independent of the gray wolf, Canadian Journal of Zoology

3575   78(12):2156-2166. © 2008 NRC Canada or its licensors. Reproduced with permission.

3576

3577   Figure 11. Distribution of control region mtDNA haplotypes of *Canis lupus* in British Columbia

3578   (Figure 3 of Muñoz-Fuentes et al. 2009). © John Wiley and Sons. Used with permission.

3579

3580   Figure 12. Median-joining network of Y-chromosome haplotypes of western wolves, western

3581   Great Lakes states wolves, and Wisconsin coyotes (Figure 4 of Fain et al. 2010). Green = *C.*

3582   *lycaon*; yellow = coyotes; orange = *C. lupus*. © Springer. Used with permission.

3583

3584   Figure 13. Summary of major conclusions of this review. Solid lines within North American

3585   indicate general boundaries of species and subspecies of *Canis* recognized by Nowak (2002).

3586   Dashed lines indicate recommended alterations or qualifications of Nowak's geographic

3587   boundaries of taxa. Geographic positions of these lines are approximate.   **A.** *Canis lycaon* is

3588   recognized as a species, rather than a subspecies of *C. lupus*. The historical boundary between

3589   *C. rufus* and *C. lycaon* is uncertain owing to absence of samples from the potential area of

3590   contact. **B.** Range of *C. lycaon* is extended westward; the extended area also includes *C. lupus*

3591   and *C. lupus* x *C. lycaon* hybrids. **C.** Extension of historical boundary of *C. l. baileyi* northward

3592   based on morphometric and genetic data. **D.** Taxonomic standing of *C. l. arctos* is uncertain. **E.**

3593   There are genetic discontinuities between Pacific Coast populations assigned by Nowak to *C. l.*

3594   *nubilus* and adjacent *C. l. occidentalis* populations; they are phylogenetically closest to and the

3595   limited number of historical samples of western *C. l. nubilus*. **F.** Current distribution of *C. l.*

3596   *occidentalis* has extended southward through both natural expansion and reintroduction.

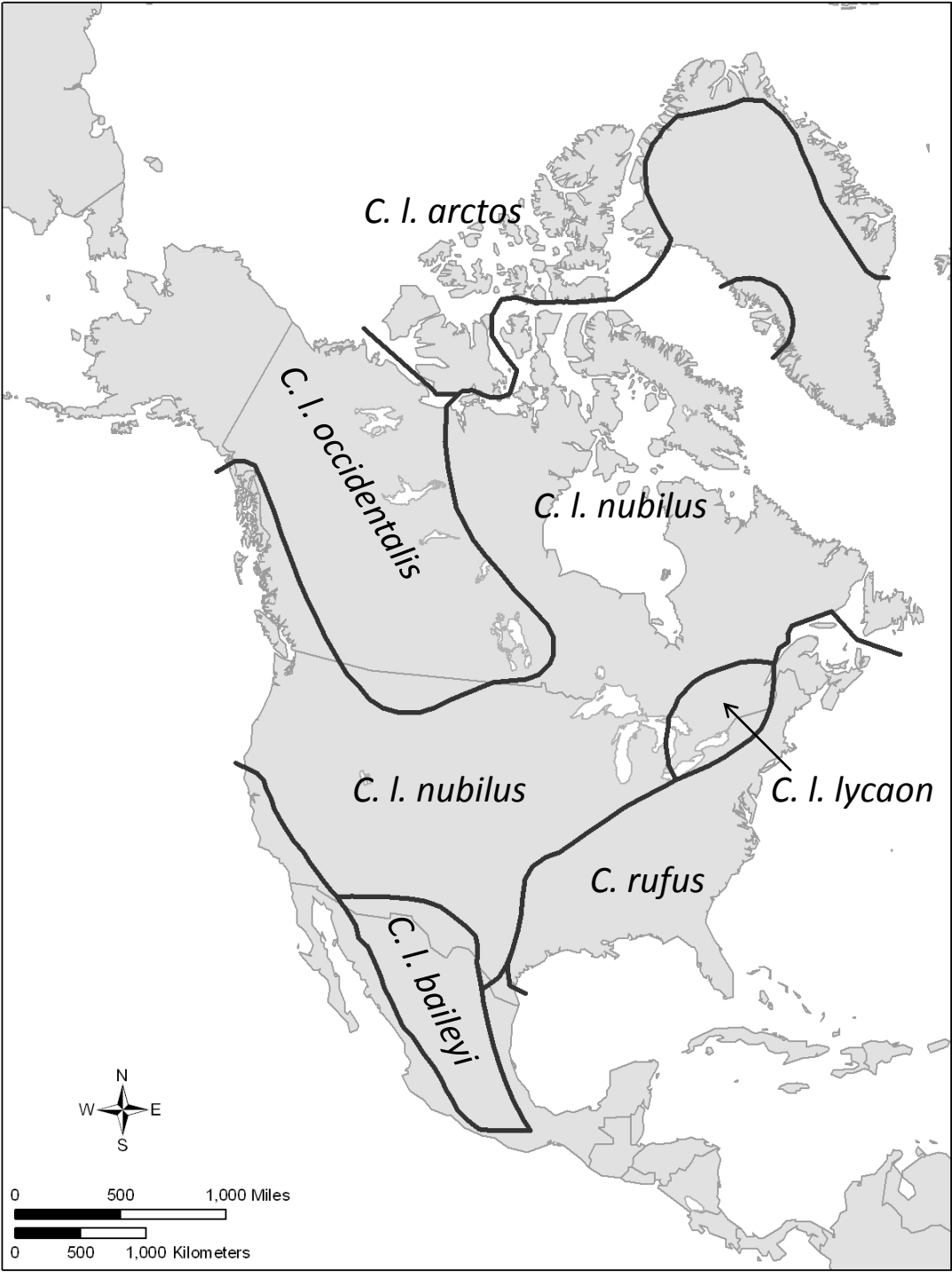3597

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3610   Figure 1.

(a)

(b)

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3614   Figure 2.

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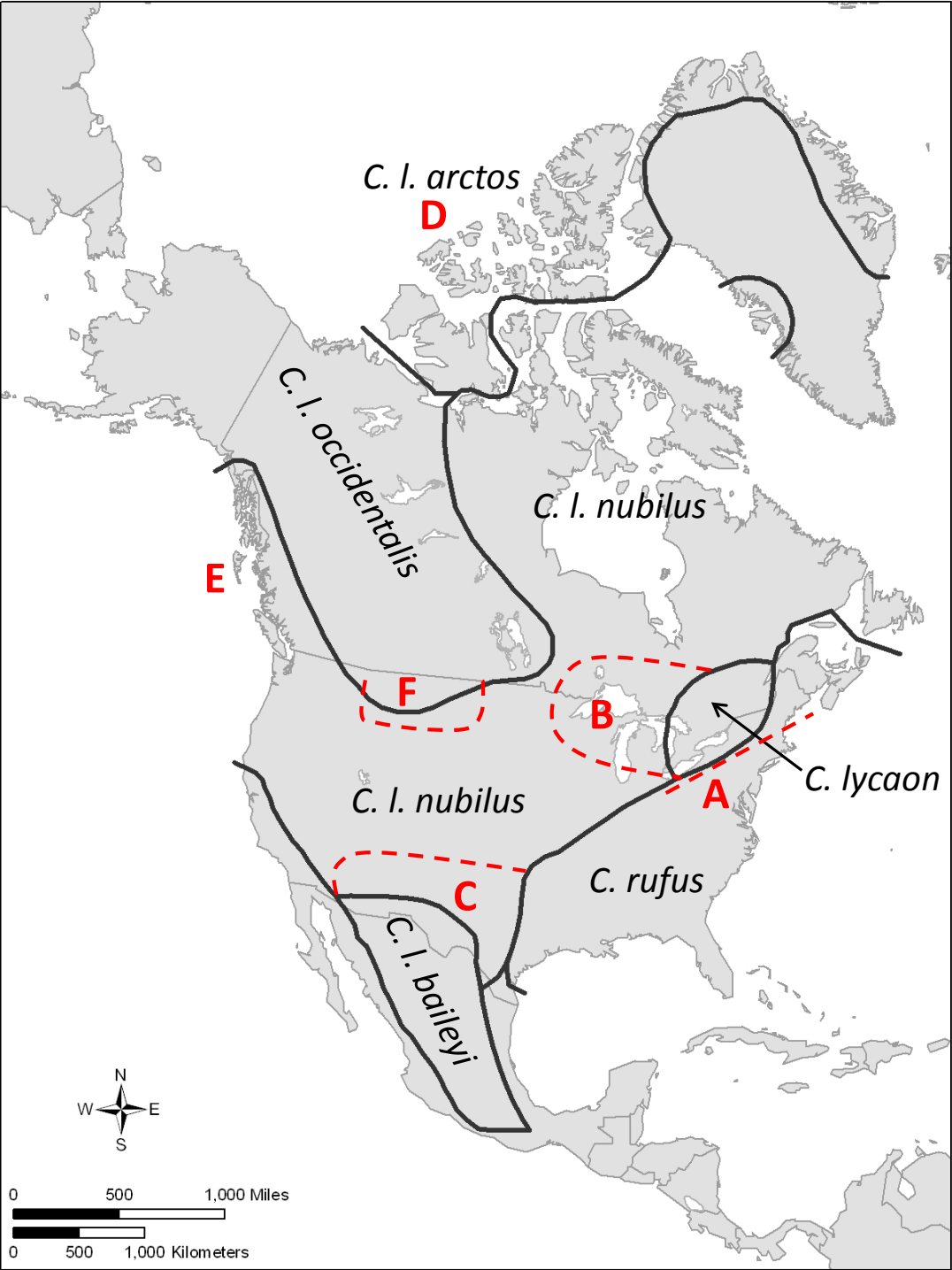3618   Figures 3 and 4 include material under copyright and are not included.

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3620   Figure 5.

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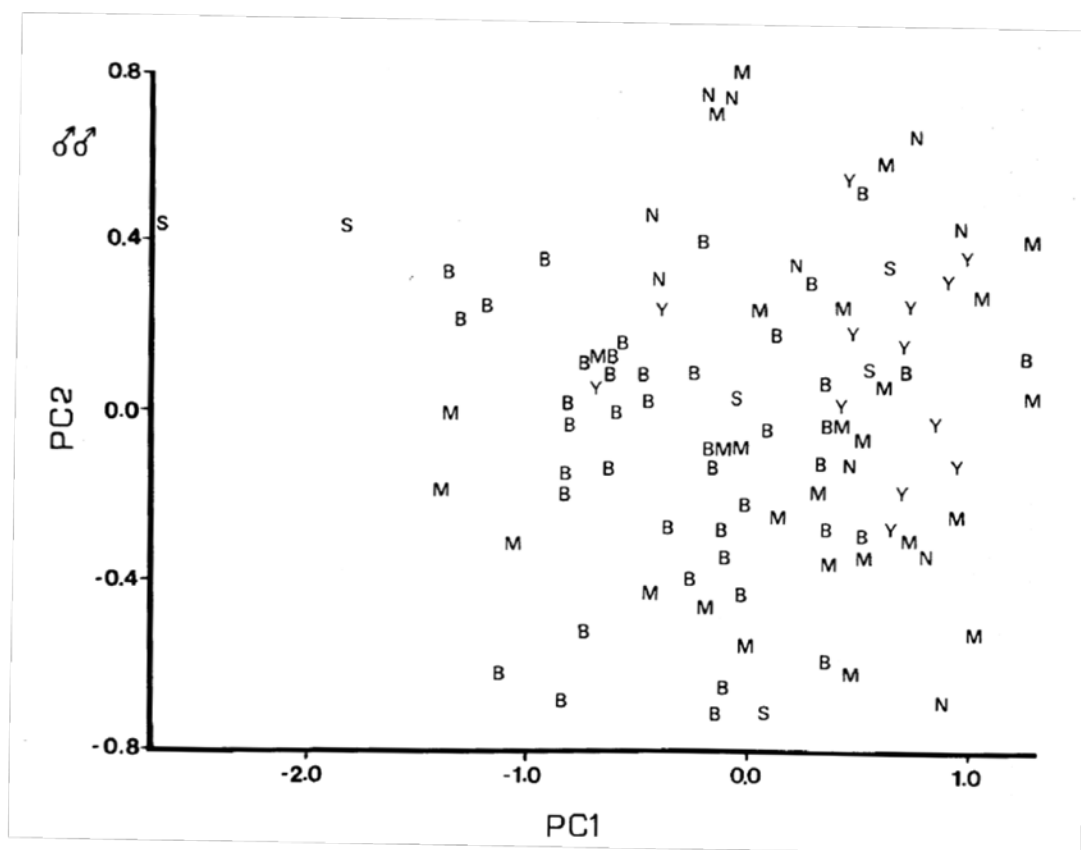
3626   Figures 6-12 include material under copyright and are not included

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3630   Figure 13.

3631